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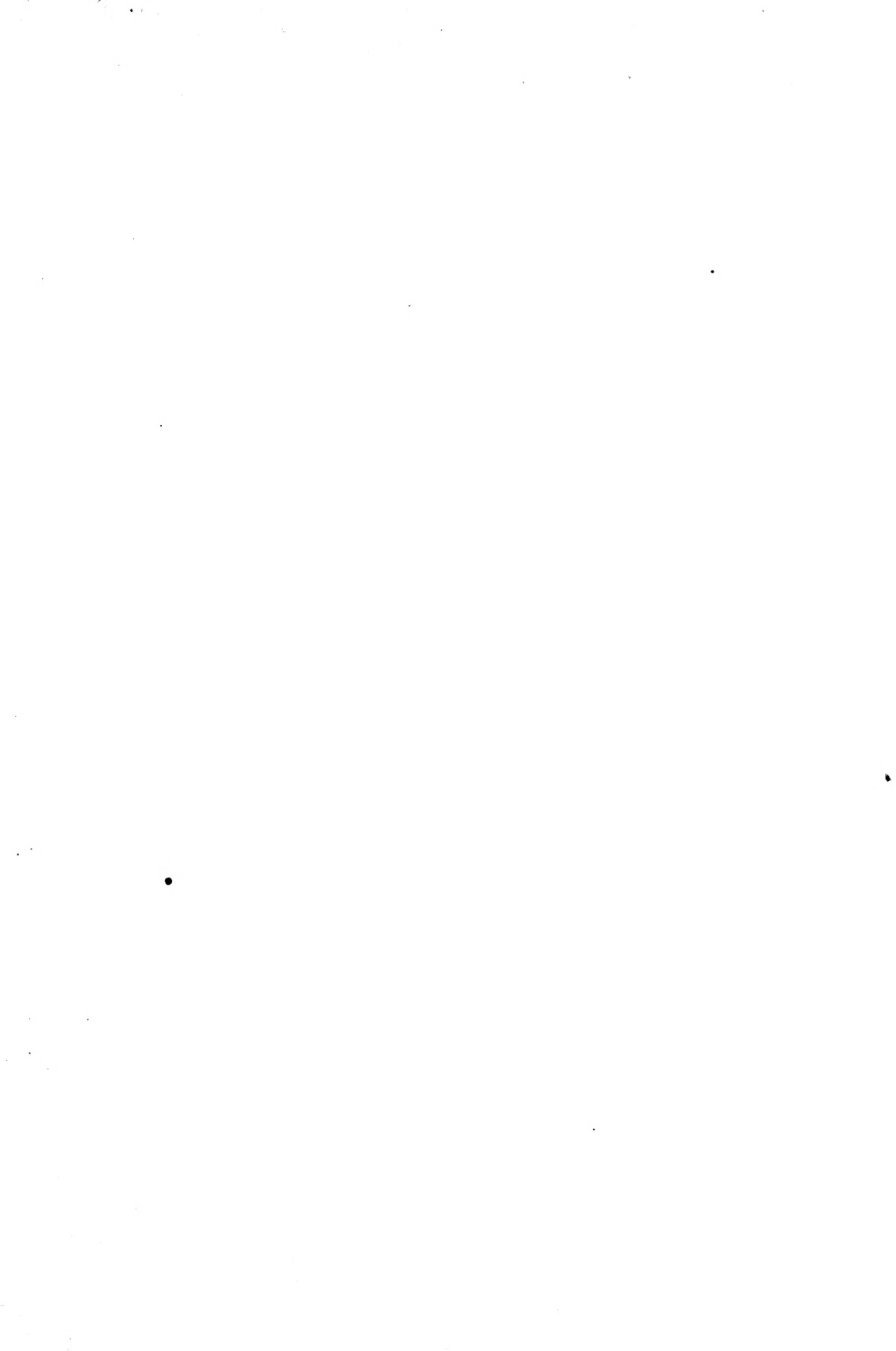
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## EFFECTS OF VARIATIONS IN ACTIVITY, FOOD INTAKE AND ENVIRONMENTAL TEMPERATURE ON WEIGHT GAIN IN THE ALBINO RAT<sup>1</sup>

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Animal forms dispose of the energy of their foodstuffs in at least three different ways: 1, as mechanical work; 2, as heat, and 3, as energy stored in the form of protein, fat or carbohydrate. The biochemical reactions associated with these energy exchanges have been studied for at least fifty years and are beginning to be fairly well worked out. Little is known, however, of the mechanisms which regulate the rate of these exchanges and which determine whether food will be stored or transformed to work or heat.

In order to study these mechanisms of regulation, it is necessary either to control or to measure the variability of the four factors—food intake, work, heat, storage—which are believed, on purely theoretical grounds, to make up the sum total of the energy metabolism of the animal. With certain precautions the food intake can be measured directly; the work output of the rat can evidently be estimated in the familiar “activity” cage; energy storage will ultimately change the body weight of the animal and can be so determined; and the energy lost in maintenance of body temperature can be controlled in normal rats by keeping the animals in a constant-temperature room. The procedures used in this investigation were based upon the foregoing considerations, as follows.<sup>2</sup>

**PROCEDURE.** Twelve female albino rats of the Sprague-Dawley strain were used continuously from the age of 4 to 10 months. In that time their body weight gradually increased from an average initial level of 142 (range of 126.5 to 153.5) to an average of 170.5 (range of 167.0 to 179.5) grams. They remained in excellent condition and showed no evidence of dietary deficiency other than the slow rate of gain in weight. They were fed a diet of finely ground “Purina Chow” mixed with an equal weight of water; weighed amounts of this food were given morning and night. Spilled food was weighed periodically; an

<sup>1</sup> Aided by a grant from the Fluid Research Fund of the Yale University School of Medicine.

<sup>2</sup> It is proposed also to present the technique in greater detail elsewhere in a paper entitled, “The use of ‘activity’ cages in quantitative metabolic experimentation.”

equivalent amount was then added to the next feeding. Spontaneous activity was estimated by means of "Veeder" counters connected to round cages fastened to horizontally mounted bicycle hubs. Water was always present in a small "living" cage adjacent to each "activity" cage. All experiments were done in a constant-temperature room where the rate of circulation of air was also constant. Weight changes were followed by averaging the values obtained by weighing the rats at the morning feeding time of two consecutive days—the last day of each five-day period and the first day of the next period.

Data have been represented graphically by plotting activity (average number of revolutions per day per 5-day period) against weight change (grams per 5-day period). Each point on a graph represents the weight change and activity of one rat for one 5-day period. Regression lines have been drawn by inspection (broken lines) and by the method of least squares (solid lines).

**RESULTS. Activity and weight gain.** When environmental temperature and food intake were constant, a negative correlation was found between activity

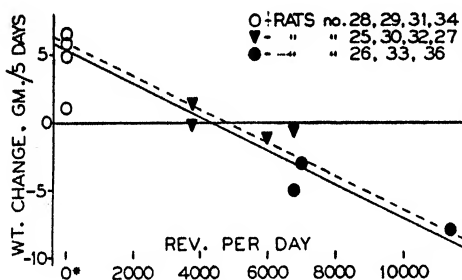


Fig. 1

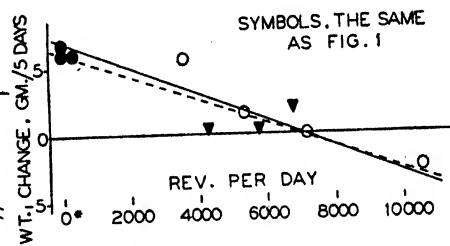


Fig. 2

Figs. 1 and 2. Negative correlation between weight change and activity in female rats on a constant food intake in a constant-temperature room. \* Cages locked.

and weight gain (figs. 1 and 2). Under the conditions of these experiments, rats in cages locked to prevent rotation gained about 5 grams/5 days, while active rats lost as much as 5 grams/5 days. It was possible to make the rats gain or lose at will by locking or unlocking the cages.

**Food intake and weight gain.** When weight change was plotted against activity, the position of the resulting curve upon the graph was observed to vary with the food intake of the rats when environmental temperature was constant. An increase of 15 per cent in the food intake elevated the weight gain of inactive and active rats by about 5 grams/5 days (fig. 3), while an increase of 25 per cent effected a proportionately greater augmentation (fig. 4). Under the conditions of this experiment, therefore, the weight gain varied with the food intake when the environmental temperature and activity were constant. (On figs. 3 and 4 the terms "Diet A" and "Diet B" identify two separate shipments of chow, the calorific values of which were evidently unequal as measured by this technique.)

**Environmental temperature and weight gain.** When weight change was plotted

against activity, the position of the resulting curve upon the graph varied with the environmental temperature when the food intake was constant (fig. 5). Elevation of the room temperature from 70° to 86°F. doubled the weight gain of inactive rats and added about 5 grams/5 days to the expected weight change of active animals.

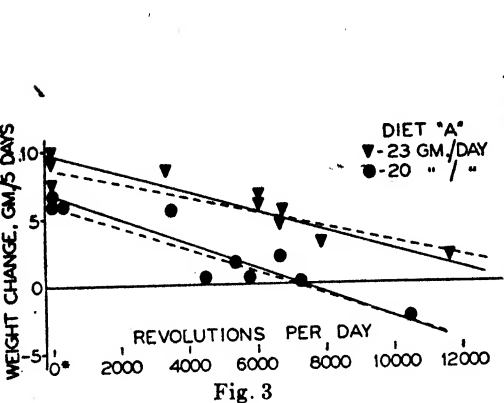


Fig. 3

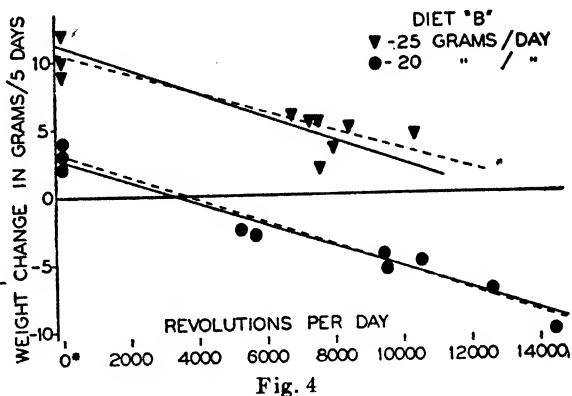


Fig. 4

Fig. 3. Negative correlation between weight change and activity in female rats in a constant-temperature room before and after the food intake was increased by 15 per cent.

\* Cages locked.

Fig. 4. Same as figure 2, before and after the food intake was increased by 25 per cent.

\* Cages locked.

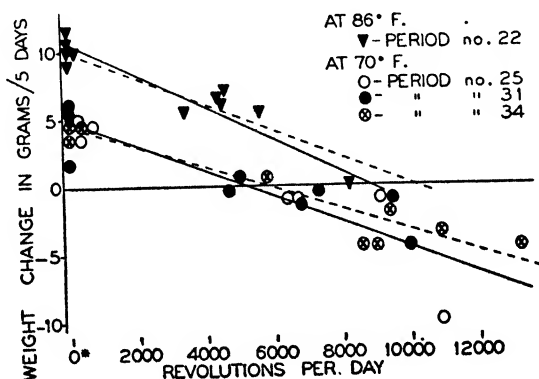


Fig. 5. Negative correlation between weight change and activity in female rats on a constant food intake at 70° and at 86°F. \* Cages locked.

*Consistency of the data.* Figure 5 also gives some indication of the consistency of these data since it includes the results of 3 different experimental periods extending throughout an interval of 50 days (periods 25, 31 and 34). On the vertical axes of the graph most of the points are within  $\pm 3$  grams of the regression line; this deviation, if it represents the error in obtaining the true weight of the animals, is an experimental error of  $\pm 2$  per cent in weighing rats of about 150

grams. The magnitude of the changes produced by altering activity, food intake or environmental temperature appears to be well outside the range of this variation.

Although 12 rats were used throughout, many of the graphs include fewer than 12 points because of the omission of points determined within the first 5 days after a given cage had been either locked or unlocked. Whenever feeding habits or temperature regulation was found to be disturbed in a rat, the data from that period were likewise discarded. The significance of changes in the slope of the regression lines is not clear because the experiments are as yet too few in number conclusively to establish the true slopes of the lines.

DISCUSSION. These experiments suggest that food intake, activity and temperature regulation in the rat are in a certain sense independent variables upon which weight gain depends. When there is an excess of energy supply over expenditure, storage occurs; if there is no surplus, the animal apparently does not create one by lowering its body temperature or by decreasing its locomotor activity. For example, under conditions where rats in locked cages consistently gained about 1 gram/day, animals in unlocked cages ran enough to bring about persistent weight loss.

Since normal animals are obviously able to regulate food intake, activity and body temperature, these experiments also suggest that this technique may be used in attempting to localize the regulatory mechanisms. Disturbances in the regulation of body temperature, (1) of activity, (2) and of food intake, (3) have already been reported in experimental animals with hypothalamic lesions. The hypothalamus, therefore, may prove to be the level of the central nervous system at which integration of the control of energy exchanges takes place.

The energy relationships described here are in complete agreement with the principles of thermodynamics, and could have been rather fully predicted from the calorimetry and oxygen consumption studies of Rubner, Benedict, Lusk, DuBois and many others. In contrast to the usual calorimetric experiment, however, the present series represents an attempt to study over relatively long periods of time the sum total of the energy exchanges of animals while they pursue their usual laboratory routine of eating, drinking, activity, rest, waking and sleeping. By means of the technique outlined here it is relatively easy to perform a type of balance experiment which otherwise requires facilities for 24-hour direct or indirect calorimetry under conditions where it is difficult to measure spontaneous locomotor activity.

#### SUMMARY

The following relationships have been observed in the energy disposition of female albino rats:

1. There was a negative correlation between weight change and activity when food intake and environmental temperature were constant.
2. Increasing the food intake increased body weight gain when activity and environmental temperature were constant.

3. Weight gain was greater at 86° than at 70°F. when food intake and activity were constant.

Since the regulation of body temperature, of activity, and of food intake has been shown to be disturbed in animals with hypothalamic lesions (see references), the hypothalamus may be the level of the central nervous system responsible for control of energy exchanges.

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# PERIODIC FLUCTUATIONS IN THE DARK ADAPTED THRESHOLD<sup>1</sup>

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Measurements of the course of dark adaptation by the method of flash perception are usually represented by smooth curves which drop rapidly and steadily toward an asymptote parallel to the time axis. These smooth curves are drawn so that the data are distributed symmetrically about it and the variations are attributed to random error. Good examples of this method of presentation may be found in the work of Hecht and colleagues on dark adaptation (Hecht et al., 1937).

When the terminal dark adapted threshold is measured by repeatedly presenting the stimulus (flash or form) at a given brightness, it is found that the number of correct responses depends on the brightness level at which the stimulus is presented. The curve obtained by plotting the number of correct responses against the logarithm of the brightness of the test field is known as the "frequency-of-seeing" curve. As the brightness level of the stimulus is increased the "frequency-of-seeing" curve ascends continuously from the asymptote at which no correct responses are given to the asymptote at which correct responses are always given. Hecht (1942) explains the existence of the "frequency-of-seeing" curve on the basis of the number of light quanta reaching the eye. Other investigators have referred to an instantaneous probability of seeing existing as a continuous function of the stimulus brightness without ascribing any underlying mechanism.

Allen (1929) postulated what he termed the "The Neural Oscillatory Effect." It is his conception that a stimulus applied to a sense organ generates nervous impulses which are conducted to the nerve centers. These nerve centers in consequence release energy in the form of efferent impulses which are conducted back to the stimulated sense organ. The magnitude of response of the sense organ to a second stimulus depends upon the phase of the neural oscillation cycle which is at the moment predominant.

Allen considered that his data presented evidence that the corresponding nervous systems on the two sides of the body may oscillate in opposite phases as well as in the same phase. The responsiveness of one side may be depressed while that of the other side is enhanced, with resulting phenomena of neural interference, reinforcement and resonance.

The authors of this paper have observed periodic fluctuations in the dark adaptation curves of a number of human subjects. These fluctuations are especially noticeable in the terminal threshold. They have periods and amplitudes of the correct order of magnitude to explain the existence of the frequency of seeing

<sup>1</sup> The opinions and views set forth in this article are those of the authors and are not to be considered as reflecting the policies of the Navy Department.

curves. However, the periods of the fluctuations are too long to be satisfied by either the quantum or the more vague instantaneous probability hypotheses. They are observed in each eye independently and the two eyes can be in phase or out of phase. Binocular thresholds exhibit variations in amplitude of fluctuation such as would be produced by interaction between two loosely coupled, oscillating systems having slightly different periods.

**APPARATUS AND TECHNIQUE.** The data presented have been drawn from tests in a variety of experiments, few of which were designed to explore the subject of this paper. Examples which best illustrate the characteristics of the fluctuations have been deliberately chosen. About one-third of the subjects examined for various purposes exhibited the phenomenon.

The adaptometer used to make the measurements was constructed in this laboratory. The test field is a flashed opal glass plate subtending 3 degrees at the subject's eyes. It is illuminated by an incandescent source. The brightness is controlled by means of a neutral glass wedge and filters taken from a Hecht-Shlaer adaptometer. The subject controls the brightness by means of a rod, chain and sprocket arrangement. The distance from subject to test field is 4.5 feet. A synchronous motor and sector disk interrupt the light from the source producing 1.2 blinks per second of approximately equal light and dark phases. Five small luminous red dots forming a centered square are located 7 degrees above the test field and constitute the fixation pattern. Readings are taken every half minute. Starting well below the threshold the subject manipulates the brightness control until the test field is just visible, then he decreases the brightness until the test field is just extinguished. The mean of the logarithm of these two brightness readings is taken as a measure of the threshold. The booth in which the adaptometer and subject are located can be illuminated uniformly when it is desired to light adapt the subject.

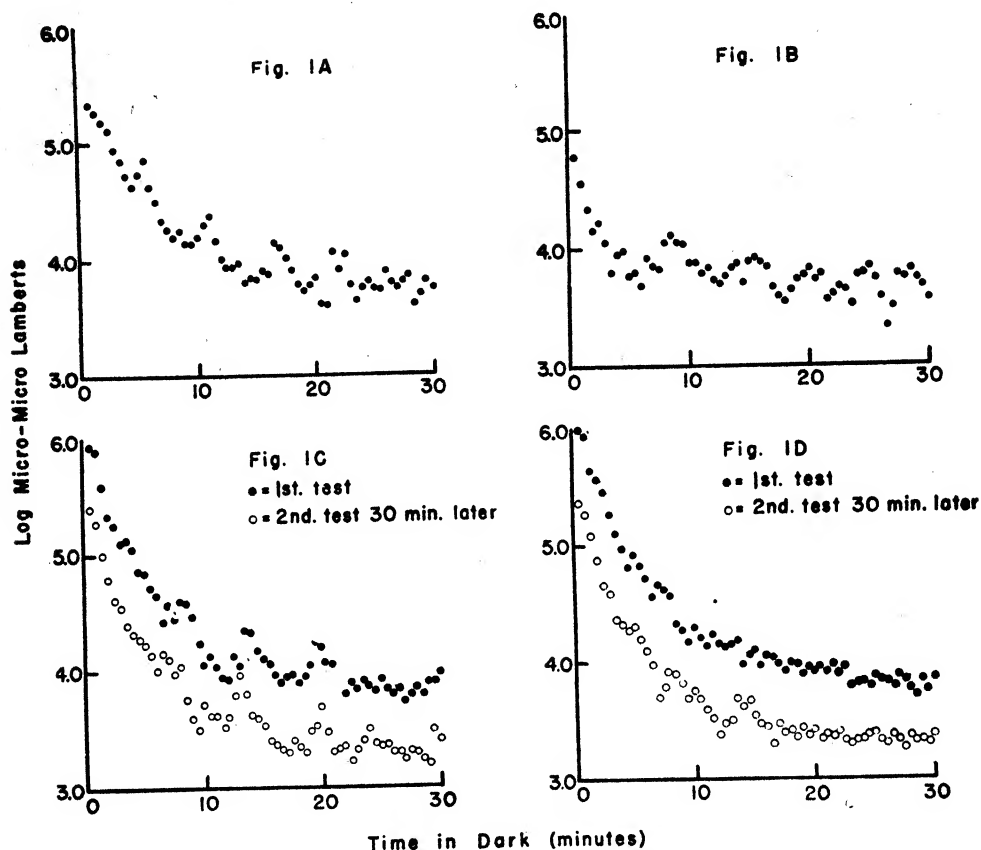
**RESULTS.** The fluctuations were first observed while the authors were measuring the effect of exposure to red light of various intensities on the course of dark adaptation (1B). The fluctuations were attributed to after images peculiar to red light exposure. Subsequent tests following preadaptation to white light of comparable intensities contained fluctuations of the same period and amplitude (1A).

It was noted that the amplitude, and to a lesser extent the period of fluctuation, varied from day to day. In order to learn whether or not the pattern was reproducible within the same test session, a series of paired tests was run with a 30 minute interval between them. Test conditions between pairs and from day to day in this series were identical. Binocular vision was used throughout. On some days both tests exhibit fluctuations similar to the examples shown (fig. 1C) while on another day the fluctuations are for the most part absent in both tests (fig. 1D). The smooth pattern is atypical for this particular individual, but for some subjects it is the customary one.

In two of the examples of fluctuations (1A and 1C) they appear to be dying out toward the end of the test period. In a binocular test in which a subject had been dark adapted for 30 minutes before the beginning of the test, however, the



fluctuations were negligible during the first 5 minutes, but then increased in amplitude and persisted during the remaining 25 minutes (fig. 2A). Monocular measurements of the threshold for the same subject, W., (fig. 2B) show an oscillation



Curves showing fluctuations in the threshold during the first 30 minutes of dark adaptation.

Fig. 1A. Subject L., 3/4/43. Following exposure to white light of 25 millilamberts brightness.

Fig. 1B. Subject B., 8/9/43. Following exposure to red light.

Fig. 1C. Subject F., 3/28/44. Following exposure to white light of 25 millilamberts brightness. This exposure was repeated 30 minutes later and the second test is charted 0.5 log units below the first.

Fig. 1D. Subject F., 3/23/44. Same exposures and procedure as in figure 1C., above.

of the same period and amplitude as those taken binocularly. Data obtained when measurements are taken alternately with the right and left eyes show that the two eyes may be in (first 20 min.) or out (last 10 min.) of phase (fig. 2C). The binocular response (fig. 2D) might be explained by an interaction between two eyes out of phase interfering destructively with each other (in a sequence similar to that appearing in fig. 2C).

Fluctuations also occurred in the dark adaptation curves when subjects were tested by means of apparatus, techniques, and under conditions other than those specifically described above. These conditions are listed below without illustration or discussion:

a. Mydriasis produced by paradrine, apparatus and test conditions as described in this paper.

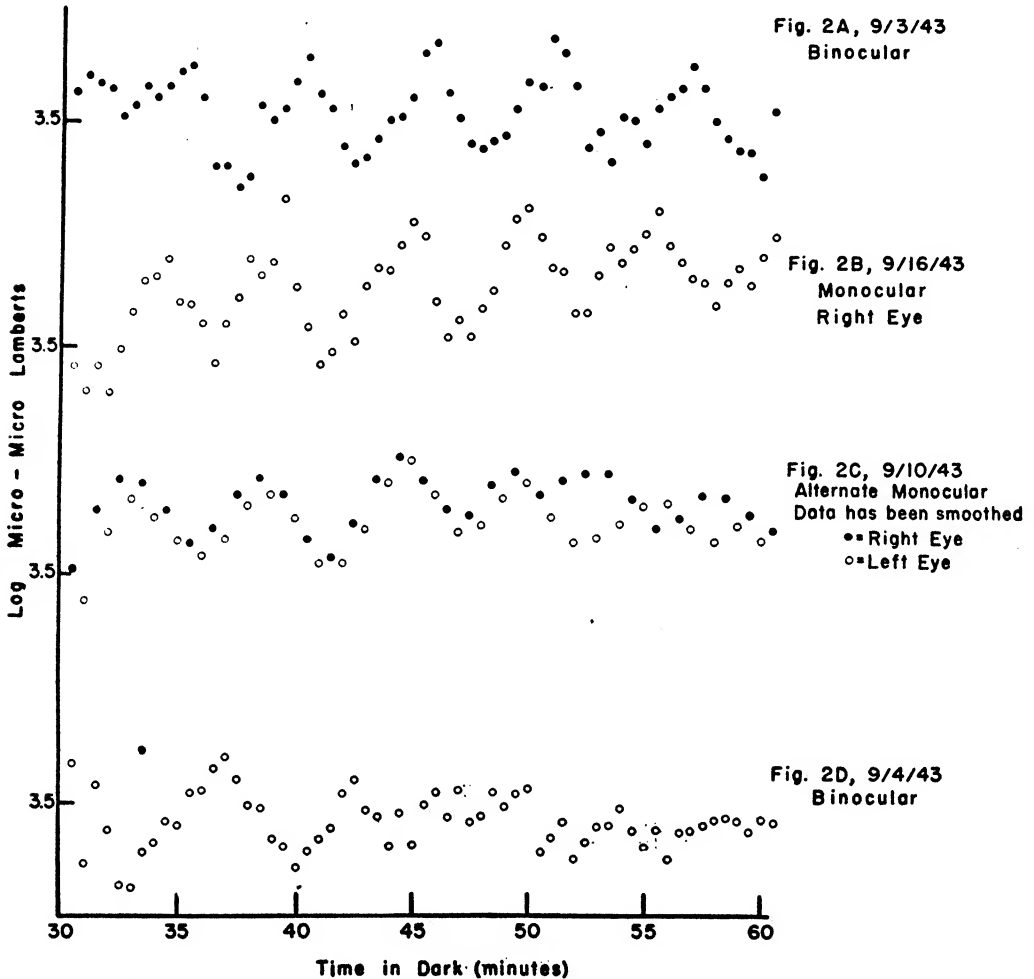


Fig. 2. Thresholds for subject W. after 30 minutes in dark

b. Adaptometer modified by using a 5 degree test field, 7 degrees above a single red fixation point. Subject responded to appearance of flash only.

c. Test conditions unchanged but sector disk removed. Subject maintained brightness at the threshold.

d. Adaptometer same as described in body of paper but a different sector disk substituted which gave a very small light-to-dark ratio (roughly 1/5 sec. light to 1 sec. dark).

e. Hecht-Sehlaer adaptometer (one subject) conditions: 3 degree test field, 7 degree fixation, 1/5 second flash.

#### SUMMARY AND CONCLUSIONS

1. Periodic fluctuations have been observed in the dark adaptation curves of approximately one-third of the subjects tested in this laboratory during the past year. These fluctuations are especially noticeable near the terminal threshold and may persist without diminishing for at least an hour after exposure to light.

2. The magnitude of the fluctuations is sufficient to account for the "frequency-of-seeing" curves obtained when dark adaptation is measured at the terminal threshold by repeated stimuli and the number of correct responses related to the stimulus brightness.

3. The period of the fluctuations is too long to permit explanation by either the quantum hypothesis of Hecht or by any type of instantaneous probability hypothesis.

4. Amplitude and period of fluctuation may vary from day to day but are remarkably similar when the same subject is tested twice with a half-hour rest period between tests.

5. Binocular tests usually show changes in amplitude of fluctuation during the course of the test. These changes are much less noticeable in monocular tests or in tests in which each eye is tested alternately (alternate monocular tests).

6. The fluctuations observed in one eye may be in or out of phase with those observed in the other eye.

7. Comparison of binocular and alternate monocular tests indicate that interaction between the two eyes may produce the changes in amplitude of fluctuation observable in binocular dark adaptation tests.

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# A STUDY OF ORTHOSTATIC INSUFFICIENCY BY THE TILTBOARD METHOD

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Marked individual differences are now known to exist in resistance to the circulatory failure induced by the centrifugal forces encountered in the operation of aircraft. Poppen (1941) and Graybiel and McFarland (1941), believing that inadequacies in the cardiovascular response to changes in posture on the tiltboard leading to fainting might be associated with a tendency to "blackout" in flying maneuvers, have suggested the development of a tiltboard test for measurement of physical fitness with special reference to aptitude for this type of flying.

The data reported here are offered as a basis for standard tiltboard test. We have been particularly concerned with attempting to characterize those subjects who fainted on the tiltboard ("fainters") in contrast with those who do not ("nonfainters") by comparing various cardiovascular measures of the two groups at rest, during and following exercise, and during tilting (before fainting). We have also sought to determine whether physical training could improve the resistance of "fainters" to gravitational stress.

**METHODS.** Our tiltboard is an adaptation of the hip-suspension type described and used by Mayerson of Tulane University.<sup>2</sup> The subject is supported at the hips by a shaped, sponge rubber padded crossbar which bears on the tissues over the iliac crest, the buttocks fitting into a shallow concave depression in the plywood top extended by a rounded cross cleat to prevent slipping down. In the present series of experiments, observations were made with the tiltboard in the horizontal ("reclining") position and at a 70° angle ("tilted").

When the body weight is supported at the hips, as in this apparatus, the muscular activity of the legs occurring when a footboard is used is avoided. Accordingly, our arrangement identifies more fainters than did that of Graybiel and McFarland who used a footboard. That this result is not due to occlusion of the venous return by the pressure of the crossbar on the femoral triangles is shown by absence of subjective or objective signs of arrested circulation even when subjects on the tiltboard in the horizontal position were "overclamped".

Of the 91 subjects employed in this study, 50 were enlisted men from the Moffett Field Air Base and 41 were unselected male college students. In general, all subjects were between 18 and 28 years of age, enjoyed good health, were free of medical defect, and did not depart in any known way from a repre-

<sup>1</sup> The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and Stanford University.

<sup>2</sup> Details of the tiltboard were kindly supplied by Doctor Mayerson

sentative sample of young men. With the exception of a few afternoon experiments the tests were conducted during the period 8 to 12:30 a.m. No subject was tested within an hour and one-half after the preceding meal.

Certain personal and anthropometric data were taken. Each was then given a short cardiorespiratory fitness test and after at least thirty minutes of rest the tiltboard test was carried out as follows:

The subject was clamped on the board and immediately tilted to check the adjustment of the clamp. He was then returned to the horizontal position for 17 minutes, during the last 7 of which heart rate and blood pressure were determined. The table was next tilted to 70° and these determinations repeated every minute for 20 minutes. Careful watch was kept for signs of circulatory failure: pallor, coldness or cyanosis, "emotional" sweating, restlessness, pupillary dilatation, and marked changes in respiratory rate or depth. The subject was asked to report any symptoms such as dizziness, faintness, nausea, darkening of the visual field, breathlessness or palpitation. If the subject fainted or appeared certain to faint before 20 minutes, he was immediately returned to the horizontal position and the experiment terminated.

The subjects were questioned for history of previous fainting under any circumstances, or of dizziness, faintness, or other difficulties on arising or prolonged standing.

RESULTS. Of the 91 subjects, 20 (the "fainters") suffered acute circulatory collapse sometime before the end of the 20-minute tilting period; the remaining 71 (the "nonfainters") were able to complete the experiments. The performance of these two groups on the tiltboard will now be considered.

1. The *nonfainters'* response to tilting was characterized particularly by maintenance of heart rate and blood pressure readings at a relatively constant level during the experiment (fig. 1). This is also true for cardiac output, according to the observations of Starr and Rawson (1941) and of Mayerson (1943). The absolute level of these functions was quite variable, but, as a group, heart rates were lower and blood pressures higher than those of the fainters. Heart rates (with one exception) ranged between 80 and 90. None of the nonfainter group had a pulse pressure consistently under 20 mm. during the tilting period. Although a few of these subjects made minor leg movements, as a rule they remained completely quiet. In several, paling of the face and dilatation of the pupils occurred, but in no case were these marked.

Graybiel and McFarland (1941) classified their subjects into fainters, nonfainters and intermediates. Since only three of our nonfainters showed symptoms and signs of circulatory failure (but were able to complete the experiment without more than minor discomfort and adequately maintained heart rates and blood pressures throughout), we have adhered to the fainter-nonfainter classification. Probably the intermediate group of Graybiel and McFarland, who had the support of a footboard, would have fainted on our tiltboard.

2. The *fainters* (not always allowed to reach unconsciousness) were tilted back to the horizontal as soon as the occurrence of syncope was certain. The time from tilting to this endpoint varied from 4 to 20 minutes, the intermediate cases being fairly evenly distributed throughout the intervening period.

The behavior of fainters during tilting did not differ detectably from that of nonfainters until about 4 minutes before the endpoint. It was in this interval that such signs as pallor of the face and chest, cyanosis, yawning, compulsive restlessness, etc., appeared in marked degree. Concomitantly, notable changes in the circulatory functions were observed. These trends are represented in

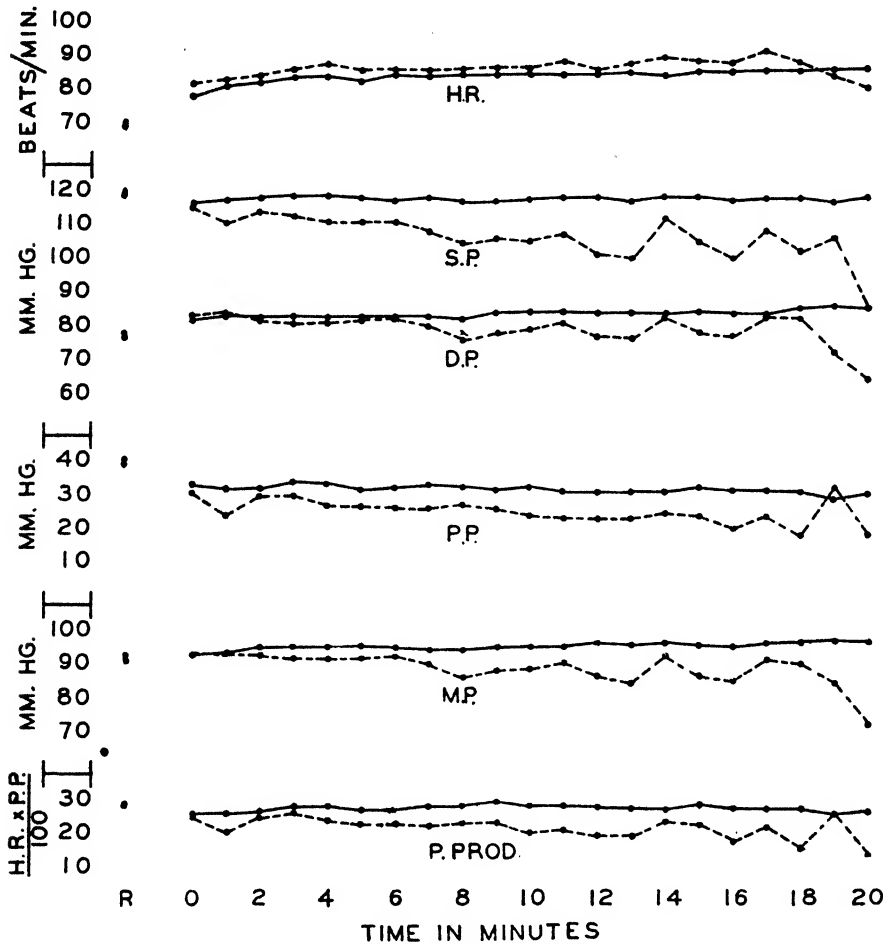


Fig. 1. Mean curves

0 --- 0 Fainters  
0 — 0 Nonfainters

figure 2, in which the values plotted at 0 were the last obtained on the subjects before fainting, those at 1 the values one minute before fainting, and so on back to a time 4 minutes before fainting. The curves have been plotted on semilog co-ordinates in order to show their relative rate of change. Since the meaningful events are distributed with respect to the time of fainting, not to the beginning of the test, and are similar whether the subject faints at 5 or 18 minutes, this method of combining the data is the only one which would preserve the significant

features of the responses. The curve of the mean response on elapsed time (fig. 1) is irregular due to the random combination, over the entire twenty-minute test period, of the various asynchronous prefainting periods.

From the fourth to second minute before fainting, heart rate and diastolic pressure remained virtually unchanged, while systolic pressure, and with it pulse pressure, underwent a pronounced fall. This drop, amounting to about 30 per cent of the preliminary value, is the most remarkable change in the functions studied. During this phase it appears that compensatory vasoconstriction was adequate to maintain the diastolic, and in large part the mean arterial pressure, even in the face of clearcut evidence of a falling cardiac output, as shown by pulse pressure and pulse product curves. (The fall in cardiac output has been directly demonstrated by the ballistocardiographic study of Starr and Rawson (1941) and by the roentgenkymographic study of Mayerson (1943).) During the next minute, 2nd to the 1st, the diastolic level dropped 8 mm. Since the systolic pressure also fell about 8 mm. the pulse pressure was unchanged, indicating that no further alteration in stroke volume occurred. In the last

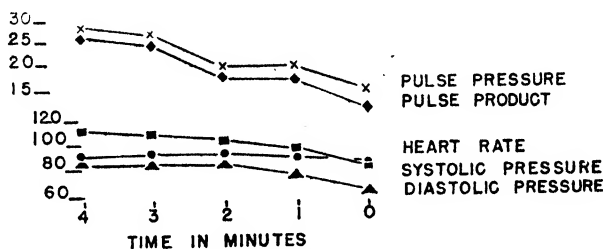


Fig. 2. Fainting trends in circulation

minute, the fall in diastolic pressure was accelerated, but that of systolic pressure, more markedly so, with the result that pulse pressure dropped to 16 mm., then approximately 50 per cent of the preliminary reading. The mean pressure (diastolic + one-third pulse pressure) was 70 mm. in the last minute.

In most cases recovery quickly followed the return to the horizontal position; occasionally it was facilitated by raising the legs above the body. In no case did any unpleasant effects persist afterward, and there is no evidence that the test is harmful.

*Correlates of tiltboard response.* 1. *Previous history of fainting* under circumstances strongly suggestive of orthostatic insufficiency was obtained from 16 of the 20 fainters, and from only 9 of the 71 nonfainters. This information, while open to obvious objection, nevertheless speaks rather for a constitutional inadequacy than for temporary indisposition of the subjects at the time of testing.

2. *Post-exercise collapse* occurring in subjects who remain standing after vigorous exercise, is doubtless the "Sportkrankheit" of Mateef (1936) and others. In this laboratory Taylor and Allen (1941) found that 17 of 100 subjects who ran to exhaustion on the treadmill displayed during subsequent standing circulatory

failure with fainting essentially similar to that occurring on the tiltboard. Of 12 subjects subjected to both tiltboard and post-exercise tests, 8 fainted in both, the remaining 4 in neither. There is thus evidence of a high correlation between the tendencies to faint under these two circumstances.

3. *Circulatory and other measures at rest and after exercise.* Since all of our subjects tested on the tiltboard had been given a submaximal exercise tolerance test, it became possible to compare fainters and nonfainters with respect to certain resting and post-exercise measures. The comparison, with measures of significance, are given in table 1. Of the measures listed therein, mean standing pulse pressure, which is smaller in fainters than nonfainters, is the only one in which these groups differ significantly. It must be concluded that neither the

TABLE 1

*Comparison of tiltboard fainters and nonfainters on certain resting and post-exercise functions*

	AVERAGE VALUES			
	Fainters	Nonfainters	Difference	P*
Age.....	22.5	22.1	0.4	610
Weight (kilos).....	73.5	70.6	2.9	246
Height (cm.).....	178.0	175.8	2.0	208
Post-ex. heart rate.....	124.1	118.3	5.8	112
Post-ex. breath-holding.....	18.4	17.8	0.6	624
Standing heart rate.....	90.3	90.1	0.2	944
Standing systolic.....	124.1	129.8	-5.7	93
Standing diastolic.....	80.0	79.4	0.6	826
Standing pulse press.....	44.1	50.1	-6.0	20
Reclining heart rate.....	73.2	71.0	2.2	374
Reclining systolic.....	122.1	122.2	-0.1	968
Reclining diastolic.....	81.0	79.0	1.9	162
Reclining pulse press.....	41.2	43.2	2.0	254
Treadmill score.....	10.2	11.1	-0.8	390

\* Times in 1000 that such a difference could have arisen from chance, obtained from the ratio of difference to its standard error.

measures taken in the resting state nor after mild exercise, used singly or in combination, serve to predict whether a subject will faint on the tiltboard.

All of the subjects also ran to exhaustion on an inclined treadmill. The scores for this performance were 10.25 for the fainters and 11.10 for the nonfainters. Since the difference could occur about once in three times by chance, it is not significant for this group. Thus, the tolerance for maximal exercise is not closely enough related to the tendency to collapse on the tiltboard to be used to predict fainting.

4. *Prefainting response on the tiltboard*, if it could be used to predict the occurrence of collapse, might permit the development of a short form of the tiltboard test. The changes in heart rate and blood pressure upon rising to a standing position after a period of reclining rest, collectively constituting the Crampton reaction (Crampton, 1905), are induced on the tiltboard although in modified



form since the active assistance of weight-bearing muscles to venous return is eliminated by the hip suspension. Nevertheless the heart rate and blood pressure respond to tilting in the same manner as in the Crampton test. The mean values of the changes in these functions during each of the first three minutes of tilting are given for fainters and nonfainters in table 2.

The slightly greater rise in heart rate, greater fall in systolic and pulse pressures, and smaller rise in diastolic pressure shown by the fainters are adverse signs

TABLE 2

*The average heart rate and blood pressure responses of fainter and nonfainter groups to the first four minutes of tilting*

	RECLINING	CHANGE ON TILTING			FOURTH MINUTE
		1st min.	2nd. min.	3rd min.	
Heart rate					
Fainter .....	73.3	11.0	12.5	13.5	89.5
Nonfainter .....	71.0	8.9	12.2	13.6	84.8
Systolic					
Fainter .....	122.0	-4.0	-8.8	-5.8	116.1
Nonfainter .....	122.2	-3.1	-2.6	-2.2	119.0
Diastolic					
Fainter .....	80.9	4.1	5.4	4.0	85.6
Nonfainter .....	79.0	5.1	6.2	6.8	83.8
Pulse press.					
Fainter .....	41.1	-8.1	-14.2	-9.8	30.5
Nonfainter .....	43.2	-8.2	-8.8	-9.0	35.2

according to the rating of these items both in the Crampton test and in the Schneider index (Schneider, 1920). The average scores on these tests are:

	Crampton	Schneider	
		HR	SP
Fainters .....	50	2	-1
Nonfainters .....	60	2	0

The ten point difference in Crampton score, in the light of the large and unaccountable variation in results usually found with this test, is without practical significance. Likewise, differences in Schneider scores are too small for confidence.

Even at the fourth minute, chosen on the basis of the data of figure 1 as the most suitable time for contrasting fainters and nonfainters, the differences between the mean circulatory values (given in table 2) are not significant, since they could occur 82 times in 1000 by chance. Accordingly, the conclusion must be drawn that in this group heart rate and blood pressure data taken early in the tilting period give no basis for the prediction of fainting.

*Prevention of tillboard fainting by leg bandaging.* Pooling of the blood in the abdomen and legs is in part responsible for the circulatory failure in orthostatic

syncope; thus, Turner, Newton and Haynes (1930) reduced tiltboard fainting by the use of tight abdominal bands, while Mateef and Schwartz (1935) prevented "Sportkrankheit" by bandaging of the legs, and Brogdon and Hellebrandt (1939) avoided orthostatic syncope by immersion of the subject in water up to the waist.

The response to bandaging was shown in one subject who fainted after 3½ minutes of tilting. He was then returned to the horizontal, and after one leg was bandaged, tilted again. After 9 minutes, during which there was no tendency toward fainting, the bandage was removed, this bringing on collapse in 4 minutes. This, and similar cases, leaves little doubt that increased leg volume is an important factor in the production of orthostatic circulatory failure.

*Prevention of tiltboard fainting by physical training.* Certain observations in the literature suggest that physical training can improve the tolerance of fainters to standing. Turner (1927) found that physical education students responded better to the prolonged standing required in her test than did other unselected female subjects, while Graybiel and McFarland (1941) noted improvement in the response of a subject following a six-month physical training program.

Identical twins, W. G. and M. G., who were discovered to be tiltboard fainters, were engaged for study of this matter. They were wiry and athletic but not outstanding physical specimens. Their medical history revealed no important diseases or defects, and their health was good.

At the beginning of the experiment, which lasted almost three months, W. G. fainted after 4 minutes of tilting, M. G. after 3 minutes. M. G. was then put on a training program consisting of graduated abdominal and trunk exercises. After one week of this regime he fainted on the tiltboard at 6 minutes, after two weeks at 12 minutes, and after three and four weeks went through the twenty-minute tilting period without fainting. Meanwhile his brother, W. G., serving as a control, fainted after one week at 6 minutes, after three weeks at 10 minutes, and after four weeks at 7 minutes. Thus, M. G. became a nonfainter while W. G., living under conditions practically identical except for the absence of specific physical training, remained a fainter.

At this point W. G. was put on the training program; after one week he fainted at 14 minutes, after three weeks at 17 minutes, after 5 weeks at 11 minutes, while after 6 weeks, not at all. The beneficial effect of training is again in evidence although M. G. improved at a slower rate than his brother.

Meanwhile the training (both the specific program and the classwork in physical education) of W. G. was discontinued. In weekly tiltboard tests conducted over the period of six weeks he continued to endure twenty minutes' tilting without fainting. Thus we have no evidence for regression during this time of the improved circulatory adaptation gained by appropriate physical training.

A similar result was obtained on another subject (J. W.), who originally fainted on the tiltboard after 3 minutes, but who during a three-weeks program (badminton, rope skipping and abdominal exercises) gradually increased the time and finally reached the criterion required for classification as a nonfainter.

It is notable that none of our three subjects could be regarded as below average according to usual standards of fitness. All were active and by no means sedentary in habit. As has been noted above, tendency to faint on the tiltboard or while standing is not closely related to exercise tolerance. One must conclude that the physical training prescribed in our experiments counteracted the orthostatic weakness in a rather specific manner.

**DISCUSSION.** One of the purposes of this study was to determine the incidence of orthostatic insufficiency in a population of young men pronounced both medically and physically fit by ordinary standards. Twenty-two per cent (20 in 91) of the college and Army subjects tested on the tiltboard fainted during the, twenty-minute experimental period. Of 20 medical students similarly studied on the tiltboard, 20 per cent fainted. A third group was required to stand for ten minutes immediately after an exhausting run on the treadmill; 17 per cent (17 of 100) of these fainted. If it be granted that post-exercise collapse is essentially similar to tiltboard fainting, then we have 211 cases in three samples which agree in demonstrating an incidence of about 20 per cent of marked orthostatic insufficiency.

Since we have ignored those cases which showed circulatory embarrassment though short of fainting, the estimate of 20 per cent is conservative.

The only comparable estimate of orthostatic collapse incidence found in literature is that of Graybiel and McFarland (1941) who studied 91 pilot cadets on a tiltboard. Of these 9 collapsed during a twenty-minute period and 13 made a "poor response" although they did not faint. Since these workers used an ordinary examination table with footboard tilted to 65° (in contrast to our hip suspension board at 70°), it is reasonable to assume that some of their 13 cadets displaying a "poor response" would have fainted on our tiltboard. If so, their incidence would agree closely with ours.

Our efforts to discover measures of the individual which might predict fainting on the tiltboard without requiring the twenty-minute test were unavailing. Neither studies of the circulation at rest, after exercise, or during a brief period of tilting, were adequate. There is at present no reliable means of identifying fainters other than the demonstration of fainting in the tiltboard test.

Finally, we wish to emphasize that the relationship between orthostatic inadequacy and exercise tolerance is so slight that it does not satisfy statistical measures of confidence in any single investigation. Nevertheless the tendency though slight is consistent. Nonfainters have higher treadmill scores, lower resting and post-exercise heart rates, longer breath-holding times, and, in the change from reclining to tilted positions, show a lesser acceleration of heart rate and a smaller drop in systolic pressure. All of these characteristics have been shown to relate to the general fitness of the individual, by such investigators as Crampton (1905), Schneider (1920), McCurdy and Larson (1939), and Johnson, Brouha and Darling (1941). It has not been so easy, however, to develop a statistically valid and reliable formulation of these measures in a test, and undoubtedly there are even more essential measures not yet incorporated in any test. Consequently, available tests, chiefly validated by the method of

individual differences, may fail miserably in assessing the fitness of the individual as it varies from time to time.

Finally, we may consider the bearing of our results on the suggestion of Poppen and of Graybiel and McFarland that the tiltboard response be used as in selecting men with high resistance to acceleration. Since none of the procedures purporting to test physical fitness made possible prediction of tiltboard circulatory collapse, it seems improbable that they would predict a tendency to "blackout" in flying. The predictive value of the tiltboard response itself may even be doubted: extrapolation from this test, in which a force of 1 G acts for 20 minutes, to conditions in flying where a force of 5 to 7 G acts only for several seconds is hardly justifiable. Until the tiltboard responses of a group of subjects have been compared with their actual ratings, this test cannot be considered a valid measure of fitness to withstand centrifugal forces.

#### SUMMARY

Fainters comprise about 20 per cent of the 111 young healthy men tested on a tiltboard at 70° with hip suspension, and about the same percentage of such men standing quietly after running to exhaustion on a treadmill. Individuals fainting in one of these situations were very likely to faint also in the other, and to have a past history of fainting.

The course of the heart rate, and of systolic and diastolic pressures of fainters and nonfainters on the tiltboard was not significantly different until about four minutes before collapse. The first sign of circulatory failure was a fall in systolic pressure, followed after a minute or so by decline in the diastolic pressure. Both fell precipitously in the last minute. In half of the cases the heart rate either did not change or rose; in the other half it showed a pronounced fall. Evidences of cerebral anemia appeared about three minutes before collapse.

No reliable sign was afforded by the circulatory data obtained during the resting state, after standard exercise, or on the tiltboard previous to the onset of collapse which might have predicted fainting. Fainters could only be identified by carrying through the tiltboard test itself.

While the average score in a maximal exercise tolerance test on a treadmill was lower for fainters, the difference was not significant. Accordingly, the ability to meet the stress of vigorous exercise and of prolonged quiet standing are two fairly distinct aspects of physical fitness.

By means of an appropriate program of physical training lasting several weeks, fainters may develop the ability to withstand gravitational stress. This has been demonstrated on identical twins, both fainters at the onset, one of whom served as a control. Evidently the physical training counteracted the orthostatic weakness in a somewhat specific manner.

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# PHYSIOLOGICALLY EQUIVALENT CONDITIONS OF AIR TEMPERATURE AND HUMIDITY

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Haldane (1909) stated that in still air resting men cannot tolerate wet bulb temperatures above 90°F. and that when the air is saturated at this temperature, body temperatures rise until symptoms of heat stroke develop. McConnell, Houghten and Yaglou (1924) also concluded that saturated still air at 90°F. is the upper limit of the environment in which man is able to maintain heat equilibrium. Cadman (1913) found that men could not work in coal mines when the air temperature was 86°F. with 100 per cent relative humidity. Data of Dreosti (1935) indicate that the above limiting values may be too conservative. His acclimatized, heat tolerant men could do light work and maintain heat equilibrium in an atmosphere with 95°F. dry bulb and 95°F. wet bulb. Winslow, Herrington and Gagge (1937 and 1938) and Gagge, Herrington and Winslow (1937) have calculated contour lines on the psychrometric chart representing the upper limits of the zone of evaporative regulation of heat by men at rest as it is affected by air movement, temperature and humidity. At high humidities their limiting environments for resting men are about the same as those of Haldane and of McConnell et al. A series of studies was made by Houghten and Yaglou (1923), Yaglou and Miller (1925), Houghten, Teague and Miller (1926) and Houghten and Teague (1928) to determine the effects of variations of air movement, temperature and humidity on the subjective comfort of men both at rest and at work. From their data these authors have drawn contour lines of effective temperatures on psychrometric charts, each line representing conditions having equal effects on the comfort of the subjects. Gagge, Herrington and Winslow (1937) have drawn a contour chart in the zone of evaporative regulation of body temperature indicating biothermally equivalent temperatures in relation to wetted area of the skin and to air temperature and humidity.

Since the practical observations of limiting hot environments have been largely in the zone from 95 to 100 per cent relative humidity and do not all agree with each other, the present study has been made to check those values and to extend physiological observations to higher temperatures with lower humidities. Because the effective temperatures of Houghten and his colleagues are based on judgment of comfort and the biothermally equivalent temperatures of Gagge

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et al. are based to a considerable extent on mathematical treatment of physical data we have attempted in this study to establish experimentally an evaluation of the effects of various environments based entirely upon the heat regulatory responses of men. We have determined the effects of the environment on clothed and unclothed men both at rest and in walking on the treadmill.

PROCEDURE. Five men were used as subjects in this study. These men were all in good physical condition and well acclimatized to work in the heat. Acclimatization was produced by having the men work for two or more hours each day in a room where high atmospheric temperatures were maintained. At

TABLE 1

*Characteristics of the men used as subjects in this study*

SUBJECT	AGE	HEIGHT	WEIGHT	BODY SURFACE
	yrs.	cm.	kgm.	m <sup>2</sup>
SR	41	171	65.5	1.76
AJ	26	181	67.5	1.86
MS	23	186	72.5	1.97
LG	23	175	63.2	1.77
BS	24	182	67.0	1.86

TABLE 2

*Comparison of the  $E_p$  values on two clothed men walking on the treadmill at a MR of 189 Cal./m<sup>2</sup> per hour. The two men walked simultaneously in experiments at each environmental condition*

SUBJECT	$E_p$	ROOM AIR °C.	
		Dry bulb	Wet bulb
AJ	176	38.0	28.4
LG	148		
AJ	141	40.5	27.0
LG	150		
AJ	149	40.6	27.2
LG	130		
AJ	156	45.0	27.2
LG	162		

least a month was used to complete the acclimatization of each man. Table 1 shows the characteristics of the men.

All experiments were carried out in an air conditioned room with an air movement of 55 meters per minute. Atmospheric temperature and humidity and the temperature of the room surfaces remained constant during each experiment, but from experiment to experiment the environment varied from cool to extremely hot. The following atmospheric conditions were used: 23°C. with 29, 53 and 76 per cent humidity; 28°C. with 27, 55 and 85 per cent humidity; 33°C. with 19, 40, 68 and 95 per cent humidity; 38°C. with 16, 42, 61, 76 and 86 per cent humidity; 45°C. with 13, 26, 42 and 58 per cent humidity, and 50°C. with 15, 27, 35 and 44 per cent humidity. At air temperatures between 23 and 30°C. the average temperature of the walls, floor, ceiling and other objects surrounding the subject was approximately the same as the air temperature. At air temperatures ranging from 30 to 50°C. the walls and surrounding objects were cooler

than the air, the average difference grading upward to a maximum of  $1.3^{\circ}\text{C}$ . at  $50^{\circ}\text{C}$ .

Subjects LG and SR wore Army jungle uniforms made of windbreak poplin, light cotton underwear shorts, shoes and socks in most of the experiments. Subjects AJ and MS wore only shorts, shoes and socks in a complete series of experiments in all environments; for comparative purposes they wore the same clothing as SR and LG in a limited number of experiments. These four subjects were all exposed to each environment for periods of 2 to 6 hours. Subject BS participated only in some of the prolonged experiments. During each exposure the following observations were made: 1. Heart rate was measured by a cardiometer at 15-minute intervals. 2. Rectal temperature was measured by a clinical thermometer at 30-minute intervals. 3. Skin temperature was measured at 15-minute intervals by four thermocouples placed separately on knee, chest, hip and shoulder. These points are best for use with working subjects because the thermocouples will remain in place for hours. A radiometer was used to measure skin temperature of arms, chests and cheeks of the men who wore shorts and to measure the temperature of the cheeks of the clothed men. The values of skin temperature presented in this paper are weighted averages including both thermocouple and radiometer readings and they give reliable measures of differences and changes in the subjects' skin temperatures. 4. Rate of sweating was calculated from nude weights of the men taken immediately before starting and at the end of each hour of the experiment, taking into account the water intake of the men and urine output if any was voided during the experiment. In the walking experiments the men stopped 5 minutes at the end of each hour for the weighings. In a number of experiments the metabolic rates of the men were measured by collection and analysis of expired air. With very few exceptions each subject went through each of his experiments 1 to 2 hours after a light meal and at about the same time of day. A total of 170 2-hour experiments were carried out. The subjects were exposed to the various environments in three different states of activity, i.e., (1) sitting, (2) walking on the treadmill at 4.5 km. per hour on a level, and (3) walking on the treadmill at 5.6 km. per hour up a 2.5 per cent grade. Forty-two additional experiments were continued for two and one-half to six hours to determine the most severe environmental conditions in which the men could maintain thermal equilibrium in the three levels of activity. During the 2-hour experiments the subjects maintained water balance by drinking measured quantities of 0.2 per cent NaCl solution. In the experiments reported here which were continued for longer than two hours three of the four subjects maintained water balance by drinking 0.1 per cent saline while the fourth man, whose sweat characteristically contained 50 to 60 m.eq. of chloride per liter, drank 0.2 per cent saline. In all of the experiments the water drunk by the subjects was maintained at a temperature near that of the body, i.e.,  $35$  to  $37^{\circ}\text{C}$ .

**RESULTS.** From the data in the 2-hour experiments an index of the physiological effect of the environment was calculated for each man for each exposure. In calculating this index the subject's heart rate, skin temperature, rectal tempera-



ture, and rate of sweating were weighted according to the following five equations:

$$(1) \quad E_h = \frac{100}{H_2 - H_1} (H_3 - H_1)$$

$$(2) \quad E_s = \frac{100}{S_2 - S_1} (S_3 - S_1)$$

$$(3) \quad E_r = \frac{100}{R_2 - R_1} (R_3 - R_1)$$

$$(4) \quad E_w = \frac{100}{W_2 - W_1} (W_3 - W_1)$$

$E_h$ ,  $E_s$ ,  $E_r$  and  $E_w$  are the effects of the environment in each experiment on the subject's heart rate, skin temperature, rectal temperature and rate of sweating respectively.

$H_1$ ,  $S_1$ ,  $R_1$  and  $W_1$  are respectively the subject's base values of heart rate, skin temperature, rectal temperature and rate of sweating. The base values on each subject were determined in each activity in a cool environment and were used as constants in calculating the effects of all other environments on the subjects in the respective activities.

$H_2$ ,  $S_2$ ,  $R_2$  and  $W_2$  are respectively the values of heart rate, skin temperature, rectal temperature, and rate of sweating of each subject in each activity observed during exposure to the most severe environments which were tolerable for 2-hour periods. These maximal values were used as constants in calculating the effects of all other environments on the subjects in the respective activities.

$H_3$ ,  $S_3$ ,  $R_3$  and  $W_3$  are respectively the subject's heart rate, skin temperature, rectal temperature and rate of sweating during exposure to the environment being evaluated.

All of the values of heart rate, skin temperature and rectal temperature used in these calculations represent the averages of all observed values during the second hour of exposure and all rates of sweating are represented by the averages of the first and second hours of exposure.

From equations 1 through 4 the overall index of the physiological effect of the environment is derived as follows:

$$(5) \quad E_p = E_h + E_s + E_r + E_w \text{ where} \\ E_p = \text{the index of physiological effect.}$$

The calculation of  $E_p$  may be illustrated by the following example from data secured on subject LG walking with a metabolic rate of 189 Cal. per M<sup>2</sup> per hour. When he performed this grade of work in clothing in a cool environment (23°C.), his base values of heart rate, skin temperature, rectal temperature and rate of sweating were respectively  $H_1 = 95$ ,  $S_1 = 31.2^\circ\text{C.}$ ,  $R_1 = 37.4^\circ\text{C.}$  and  $W_1 = 375$  grams per hour. Values of these processes observed on him in the most severe environment in which he continued this work for two hours were respectively  $H_2 = 168$ ,  $S_2 = 38.3^\circ\text{C.}$ ,  $R_2 = 40.4^\circ\text{C.}$  and  $W_2 = 1850$  grams per hour. The

above values were used as constants in the calculations of  $E_p$  on subject LG in all experiments where he wore clothing and performed the above grade of work. In an experiment where the air temperature was 45°C. with a relative humidity of 26 per cent, he performed the above work for two hours. In this experiment his heart rate, skin temperature, rectal temperature and rate of sweating determined for the factors  $H_s$ ,  $S_s$ ,  $R_s$  and  $W_s$  were respectively 117, 35.6°C., 37.8°C., and 1210 grams per hour. Substituting in equations 1 through 5, his  $E_p$  value in this experiment may be calculated as follows:

$$(1) \quad E_h = \frac{100}{168 - 95} (117 - 95) = 30.2$$

$$(2) \quad E_s = \frac{100}{38.3 - 31.2} (35.6 - 31.2) = 62.0$$

$$(3) \quad E_r = \frac{100}{40.4 - 37.4} (37.8 - 37.4) = 13.3$$

$$(4) \quad E_w = \frac{100}{1850 - 375} (1210 - 375) = 56.6$$

$$(5) \quad E_p = 30.2 + 62.0 + 13.3 + 56.6$$

$$E_p = 162.1$$

Environmental conditions producing equal  $E_p$  values for the subjects in each activity were determined. From the data of the 170 2-hour experiments  $E_p$  values for each pair of subjects under each environmental condition were averaged. These average  $E_p$  values were then plotted against the wet bulb temperatures, and a curve was drawn to fit the points determined at each dry bulb temperature. This produced a family of curves, each of which represented at its respective dry bulb temperature, the effects of variations of the wet bulb temperature upon the  $E_p$  values. These curves were then cut at certain levels of  $E_p$  and the points transferred to psychrometric charts. The contour lines representing equal values of  $E_p$  were then drawn through these points on the psychrometric charts.

The average  $E_p$ , "index of physiological effect," plotted in relation to the dry and wet bulb temperatures and the relative humidity of the atmosphere are shown in figure 1 for the two fully clothed men, SR and LG, performing moderate work by walking on the treadmill at 5.6 km. per hour up a 2.5 per cent grade (MR 189 Cal. per M<sup>2</sup> per hour) and in figure 2 for AJ and MS who wore only shorts, shoes and socks and walked at the same speed and grade (MR 188 Cal. per M<sup>2</sup> per hour). In these figures each contour of physiological effect indicates conditions of the environment which had approximately equal effects on the working men in the respective types of clothing. It is obvious from these figures that under conditions of mild heat large changes of the environment are required to make small changes in the physiological stress as indicated by the index, and that changes in the wet and dry bulb temperatures are about equally effective in altering the index. With increasing severity of environmental heat,

the index becomes increasingly sensitive to small changes in the wet bulb temperature and less sensitive to changes in the dry bulb temperature. In comparing figures 1 and 2 in the region of mild heat it is seen that in arid conditions higher environmental temperatures were required to produce an  $E_p$  value of 50 in the clothed men than in the men wearing shorts, whereas at high humidities the reverse was true. In the entire zone of atmospheric conditions producing  $E_p$  values from 50 to 150 the clothing was physiologically a more important barrier to heat dissipation by the men in humid heat than in dry heat. In environments causing  $E_p$  values of 200 or more the corresponding physiological contour lines

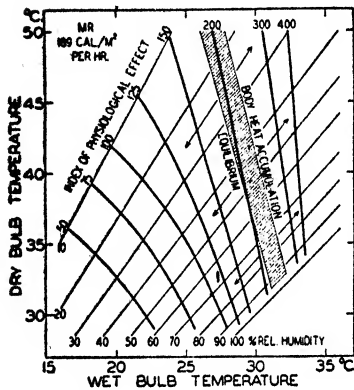


Fig. 1

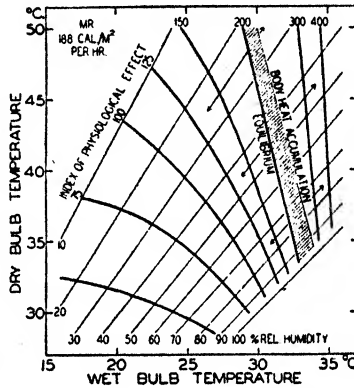


Fig. 2

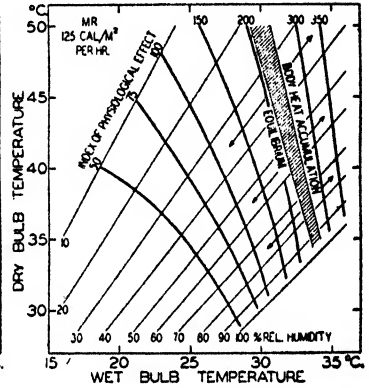


Fig. 3

Fig. 1. The physiological effects of the environment on the two clothed men walking at 5.6 km. per hour up a 2.5 per cent grade (MR 189 Cal. per  $m^2$  per hr.). Each contour line of physiological effect indicates conditions of the environment which had equal effects on the working men. Air movement 55 m. per minute.

Fig. 2. The physiological effects of the environment on the two men wearing shorts walking at 5.6 km. per hour up a 2.5 per cent grade (MR 188 Cal. per  $m^2$  per hr.). Each contour line of physiological effect indicates conditions of the environment which had equal effects on the working men. Air movement 55 m. per minute.

Fig. 3. The physiological effects of the environment on the two clothed men walking at 4.5 km. per hour on the level (MR 125 Cal. per  $m^2$  per hr.). Each contour line of physiological effect indicates conditions of the environment which had equal effects on the working men. Air movement 55 m. per minute.

of the men in shorts and the clothed men are practically parallel and show that for a given  $E_p$  the men in shorts tolerated conditions of temperature and humidity which were distinctly more severe than did the clothed men. These differences which were found between the effects of the various environments on men in shorts and on the clothed men were due largely to the clothing. A number of control experiments were run in which men who ordinarily wore shorts performed work experiments in clothes, and in these experiments their  $E_p$  values corresponded very closely to those of the regularly clothed subjects under the same conditions. This may be seen by comparing values on AJ walking in clothes with the values on LG who was ordinarily clothed (table 2). In mild to moderate

heat our contour lines of equal physiological effect on clothed men in this grade of work follow fairly closely the effective temperature lines for clothed men at work as determined by Houghten, Teague and Miller (1926). In the zone of severe conditions in which the men could not maintain thermal equilibrium our curves are more nearly parallel with wet bulb temperature lines on the psychrometric chart than are the corresponding contours of the above authors.

Figures 3 and 4 show the average  $E_p$  plotted in relation to the dry and wet bulb temperatures and the relative humidity of the atmosphere for the same four men performing light work by walking at 4.5 km. per hour on a level (MR 127 Cal.

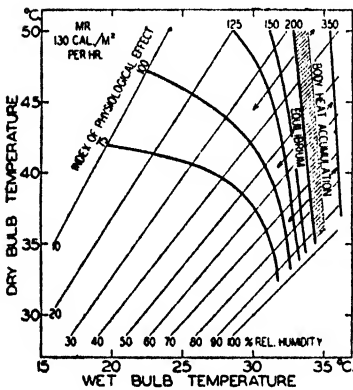


Fig. 4

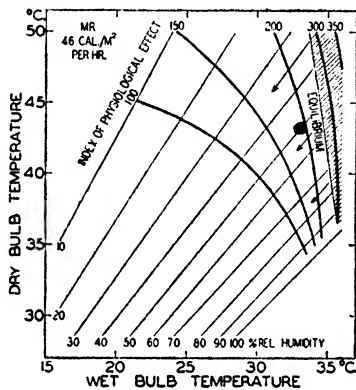


Fig. 5

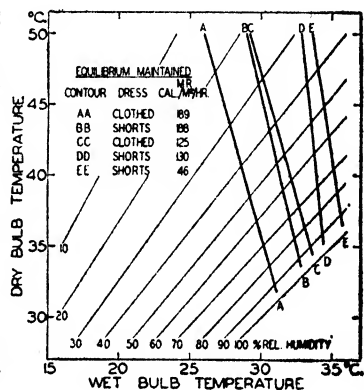


Fig. 6

Fig. 4. The physiological effects of the environment on the two men wearing shorts walking at 4.5 km. per hour on the level (MR 130 Cal. per  $m^2$  per hr.). Each contour line of physiological effect indicates conditions of the environment which had equal effects on the working men. Air movement 55 m. per minute.

Fig. 5. The physiological effects of the environment on the two men wearing shorts sitting at rest (MR 46 Cal. per  $m^2$  per hr.). Each contour line of physiological effect indicates conditions of the environment which had equal effects on the resting men. Air movement 55 m. per minute.

Fig. 6. The effects of clothing and work on the most severe environmental conditions in which men maintained thermal equilibrium from the second through the sixth hours of exposures. Air movement 55 m. per minute.

per  $M^2$  per hour). The differences between the effects of the various environmental conditions on the men in shorts and on those who were fully clothed have the same general characteristics as those described above for the moderate grade of work. The principal difference between the responses of the men in the two grades of work is that much more severe environmental conditions were required in the easy work than in the moderate work to produce equal increments of the  $E_p$ . In other words, all of the contour lines representing physiological effects in the light work are shifted toward more severe conditions of heat than the corresponding lines in moderate work. Differences between the base values of heart rate, rectal temperature, skin temperature and rate of sweating of the men in the two grades of work represent the effect of the work itself and this effect is therefore not included in the calculation of the  $E_p$  values. This shift of the  $E_p$  values

is therefore a real measure of the difference in the effects of the environment in relation to the change in the metabolic rates of the men in the two activities.

Figure 5 shows the average  $E_p$  plotted in relation to the dry and wet bulb temperatures and relative humidity of the atmosphere for 2 men sitting at rest. The principal difference between the responses of the men in shorts at rest and in work is that more severe environmental conditions were required in rest than in work to produce equal increments of  $E_p$ . As was true in comparing the two grades of work this shift of the  $E_p$  values is a real measure of the effects of the environment in relation to the change in metabolic rate of the men between work and rest, because the base values of heart rate, skin temperature, rectal temperature and rate of sweating used in calculating the  $E_p$  values were taken from experiments in work and rest respectively. In severe conditions of heat our contours of physiological effect on men at rest are approximately parallel on the psychrometric chart to the effective temperatures determined on resting men by Houghten and Yaglou (1923). In the zone of equilibrium shown in figure 5 the directions of our contours on the psychrometric chart are significantly different from corresponding contours of these authors. This difference may be associated with the fact pointed out above that in mild conditions of heat large changes of the environment are required to produce small physiological changes.

Table 3 gives representative experiments from a series carried out to determine the most severe conditions of environmental temperature and humidity in which men could maintain thermal equilibrium after the second hour of six-hour exposures. In experiments where the men clearly failed to maintain equilibrium after the second hour the minimal rate of rectal temperature rise during this time was  $0.18^{\circ}\text{C}$ . per hour. In experiments in which practical thermal equilibrium was maintained after the second hour, the greatest changes of rectal temperature were  $\pm 0.07^{\circ}\text{C}$ . per hour. In the latter experiments these small changes of the men's rectal temperatures after the second hour were negative about as frequently as they were positive. In work experiments at an air temperature of  $50^{\circ}\text{C}$ . with the wet bulb temperature maintained just above the limits of the clothed subjects' ability to maintain thermal equilibrium, the men would attain an equilibrium during the third and fourth hours and then start accumulating heat again in the fifth and sixth hours. This was because their rates of sweating declined during the experiment and they failed to keep up the evaporative requirements during the last two hours.

In figures 1 through 5 the shaded areas on the psychrometric charts are drawn to separate the zones of conditions in which the men were able to maintain practical thermal equilibrium during the second through the sixth hours of exposure from conditions in which they accumulated body heat after the second hour as described above. It will be noticed that the equilibrium zones extend throughout mild conditions of heat and approximately up to the  $E_p = 200$  contours in the two grades of work. In work  $E_p$  values of more than 250 usually fall in the zone of accumulating body heat where the men did not maintain thermal equilibrium. In figure 5 the line at the left of the shaded areas indicating conditions in which our men maintained equilibrium at rest is close and approximately parallel to

TABLE 3

*Representative experiments showing the physiological effects of moderate to severe conditions of air temperature and humidity*

The values of heart rate, rectal temperature, skin temperature and sweating are expressed as hourly averages. Air movement was 55 m. per minute in all experiments.

SUBJ.	TIME	ROOM AIR °C.		E <sub>p</sub>	HEART RATE		RECTAL TEMP. °C.			SKIN TEMP. °C.		SWEAT KG./HR.	
		Dry bulb	Wet bulb		2nd hr.	last hr.	2nd hr.	last hr.	change* per hr.	2nd hr.	last hr.	1st 2 hrs.	last hr.
Sitting in shorts MR 46 Cal./m <sup>2</sup> per hr.													
MS	hrs. 6	35.2	34.4	173	88	86	37.8	37.5	-0.07	36.4	36.1	0.71	0.48
MS	5	36.0	35.6	274	103	103	38.3	38.2	-0.03	37.2	36.5	1.00	0.76
BS	4	50.0	33.7	299	96	91	38.2	38.2	0.0	37.0	36.7	1.34	1.11
MS	3	50.0	34.8	348	107	104	38.6	38.8	+0.20	37.8	38.0	1.47	1.33
Sitting in clothes MR 49 Cal./m <sup>2</sup> per hr.													
MS	6	38.2	33.4	186	88	83	37.7	37.6	-0.03	36.6	36.1	0.87	0.39
LG	5	36.0	35.6	296	104	93	38.2	38.3	+0.03	37.8	37.2	1.07	0.66
LG	3	50.0	34.8	369	108	104	39.1	39.4	+0.30	37.9	38.1	1.43	1.23
Walking in shorts MR 130 Cal./m <sup>2</sup> per hr.													
MS	4	49.9	32.6	179	113	121	38.1	38.0	-0.05	36.5	36.5	1.35	1.16
LG	6	35.0	34.4	205	120	122	38.3	38.6	+0.07	36.4	36.4	1.32	0.42
MS	2½	36.0	35.1	271	138	136	38.9	39.2	+0.60	37.1	37.1	1.52	1.46
BS	2½	36.0	35.1	288	133	138	39.0	39.4	+0.80	36.9	36.7	1.66	2.01
MS	3	50.0	33.9	294	135	145	38.9	39.2	+0.30	37.9	37.9	1.75	1.56
Walking in clothes MR 125 Cal./m <sup>2</sup> per hr.													
MS	6	50.1	28.4	175	110	123	38.1	38.0	-0.02	36.4	36.7	1.39	1.14
LG	3	49.9	28.9	175	107	101	37.8	37.6	-0.20	36.2	35.7	1.26	1.08
SR	4	34.0	32.9	210	116	117	38.0	38.0	0.0	36.8	36.5	1.28	1.14
LG	6	34.0	32.9	224	124	119	38.3	38.2	-0.02	36.5	36.4	1.38	0.74
LG	6	34.5	33.7	242	127	123	38.6	38.7	+0.02	36.8	36.8	1.42	0.67
SR	6	50.0	31.1	262	128	134	38.5	39.3	+0.20	37.3	37.6	1.52	1.04
BS	5	50.0	31.1	280	122	135	38.6	39.7	+0.37	37.1	37.7	1.83	1.00
Walking in shorts MR 188 Cal./m <sup>2</sup> per hr.													
AJ	6	38.0	31.6	170	106	115	38.4	38.5	+0.02	35.2	35.4	1.33	0.94
MS	6	50.0	29.0	188	119	134	38.0	38.0	0.0	35.4	36.2	1.49	1.39
SR	6	34.0	32.6	196	126	130	38.3	38.5	+0.05	35.8	35.8	1.33	0.69
LG	6	34.0	32.6	193	130	136	38.3	38.4	+0.02	35.6	35.6	1.08	0.62
LG	6	50.0	30.0	194	119	134	38.2	38.9	+0.18	35.6	37.3	1.37	1.00
SR	6	34.5	33.8	238	138	149	38.5	39.6	+0.28	36.8	37.2	1.34	0.69
AJ	2	50.4	33.7	359	154		39.8		†	38.1		2.24	
Walking in clothes MR 189 Cal./m <sup>2</sup> per hr.													
LG	6	32.0	31.0	159	117	116	37.8	37.9	+0.02	35.4	35.4	1.29	0.66
LG	6	45.0	27.2	162	117	127	38.0	38.1	+0.02	35.7	35.9	1.21	0.94
AJ	6	38.0	28.4	176	101	110	38.3	38.4	+0.02	35.7	35.6	1.51	1.01
LG	6	50.0	28.0	215	133	150	38.0	39.5	+0.38	35.9	38.5	1.51	1.00
SR	6	50.1	27.4	236	135	143	38.2	38.9	+0.18	36.9	37.2	1.61	1.13
LG	2	38.1	31.0	253	136		38.8		†	36.4		1.53	
BS	2	33.0	32.3	313	151		39.3		†	36.9		1.71	
SR	2	37.8	33.8	377	165		40.3		†	38.4		1.81	

\* Change of rectal temperature in °C./hr. between the second and last hours of the exposure.

† Rectal temperature rising rapidly at the end of the second hour.

the  $E_p = 300$  contours. Figure 6 gives a comparison of the contours denoting the most severe conditions in which the clothed men and the men in shorts maintained practical thermal equilibrium for six hours. It is obvious from this graph that the severity of conditions in which they maintained thermal equilibrium was reduced significantly when the men wore clothing and was also reduced in proportion to the intensity of activity by the men.

Cadman (1913) stated that nude men cannot work in still air saturated with water at 30°C. Data of Dreosti (1935) indicate that heat tolerant natives in South Africa can perform 9,000 foot pounds of work per hour in saturated air at 35°C. This work would require a metabolic rate of 75 to 85 Cal./m<sup>2</sup> per hour. Eichna, Ashe, Bean and Shelley (1944) found that acclimatized men in shorts carrying 20-pound packs could easily walk at 3 mph for 4 hours in saturated air at 92.5°F. (33.6°C.) or in air at 120°F. (47.9°C.) with 30 per cent relative humidity. An effective air movement of approximately 3 mph prevailed during all of their experiments. In comparison with these data our men walking in shorts maintained heat equilibrium from the second through the sixth hours of exposures at 34°C. with 91 per cent relative humidity and at 50°C. with 21 per cent humidity when their metabolic rates were 188 Cal./m<sup>2</sup> per hour. At a metabolic rate of 125 Cal./m<sup>2</sup> per hour they maintained equilibrium at 35°C. with 96 per cent relative humidity and at 50°C. with 32 per cent humidity. Air movement was 55 m. per minute (2 mph) in all of our experiments. The differences in the values reported by the various groups are attributable to differences in the rate and duration of work, of air movement in the room, temperature of the walls and surrounding objects, the training and acclimatization of the subjects, and the quantity, temperature and salt content of the water ingested by the men. As explained above, in six hour exposures our men working in hot environments just above the limits of their ability to maintain thermal equilibrium, could attain an equilibrium during the third and fourth hours of the exposure and then start accumulating heat again in the fifth and sixth hours. In the experiments of Eichna et al. the mean radiation temperature of the room was always less than air temperature: 2°F. to 3°F. lower at ambient temperatures under 100°F., and 7°F. to 9°F. lower at an ambient temperature of 120°F. In our experiments the average radiation temperature of the room reached a maximum of 1.3°C. below the air temperature when the latter was 50°C. In addition, the water drunk by Eichna's subjects was not warmed to 37°C. as was that in our experiments. If a man ingests 1.5 liters per hour of water at 20°C., it will absorb approximately 26 Cal. of heat from his body. This would amount to about 10 per cent of the total heat production of a man marching at 3 mph.

The results on our resting subjects are not comparable with the data of Haldane (1909), and McConnell, Houghten and Yaglow (1924) because of differences in air movement in the experiments. In comparing the contour line of environmental conditions representing the most severe conditions in which our resting men wearing shorts maintained thermal equilibrium with a similar line calculated by Gagge, Herrington and Winslow (1937) for nude subjects at rest, we find that our contour, if extrapolated to 100 per cent relative humidity, is about 1°C. higher

than theirs at saturation with about the same air movement. However, with decreasing humidity and increasing dry bulb temperatures our limiting environments become progressively much higher than theirs. With the same air movement the upper limit of evaporative regulation of body heat on their chart is 44.5°C. with 0 per cent humidity, whereas our resting subjects maintained heat equilibrium with the air temperature at 50°C. with 34 per cent relative humidity. It must be kept in mind that these tests extended for only a few hours. It is not possible that men could permanently inhabit regions with climates so extreme as these environments which barely limit heat regulation for resting men, because the continual strain on the heat regulating mechanisms would be too great and prolonged activity would be impossible. These results prove, however, that the discomfort of people in hot weather does not ordinarily indicate a serious stress on their heat regulating mechanisms; such discomfort of most people at rest is experienced in warm environments in which they still have a fairly wide reserve in the range of evaporative cooling.

#### SUMMARY

Two hundred twelve experiments of 2 to 6 hours' duration were carried out on men in an air-conditioned room operated with dry bulb temperatures ranging from 23 to 50°C. in combination with various relative humidities. About one-half of the experiments were performed on men wearing shorts and the others on men clad in Army jungle uniforms. Three sets of exposures to the environments were made on all subjects, one with the subjects sitting, another with them walking at an easy pace on the treadmill, and the third with them performing moderate work on the treadmill.

The physiological effect of the environment in each exposure was expressed as an "index of physiological effect," by weighting equally the elevation of the subject's heart rate, rectal temperature, skin temperature, and rate of sweating from the base values of these functions determined in a cool environment on each subject in each activity. The effects of the environment on the men in relation to their activity and clothing are illustrated by six contour graphs in which their indexes of physiological effect are plotted in relation to dry bulb and wet bulb temperatures and relative humidity.

Experiments were run to determine the most severe environmental conditions in which men could maintain thermal equilibrium after the second hour of six-hour exposures. The men walking in shorts maintained thermal equilibrium from the second through the sixth hours of exposures at 34°C. with 91 per cent relative humidity and at 50°C. with 21 per cent humidity when their metabolic rates were 188 Cal./m<sup>2</sup> per hour. With metabolic rates of 130 Cal./m<sup>2</sup> per hour they maintained equilibrium at 35°C. with 96 per cent humidity and at 50°C. with 32 per cent humidity. With resting metabolic rates of 46 Cal./m<sup>2</sup> per hour the men in shorts could maintain equilibrium at 36°C. with 98 per cent humidity and at 50°C. with 34 per cent relative humidity. An air movement of 55 m. per minute prevailed during all experiments. The clothed men maintained thermal equilibrium at the respective metabolic rates only in environments distinctly less severe than those listed above for the men in shorts.



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# THE GASEOUS METABOLISM OF THE BRAIN OF THE MONKEY<sup>1</sup>

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The amount of oxygen used by the living brain, per unit of weight and time<sup>2</sup> and under varying conditions of activity, remains a matter for speculation in spite of considerable experimental study. This can be seen from the following list of representative values for the  $Q_{O_2}$  of brain found in the literature:

<i>In vivo</i>		$Q_{O_2}$
Alexander et al. (1).....	Dog	39
Handley et al. (9).....	Dog (perfusion)	32.7
Gayda (8).....	Dog	30.0
Rein (17).....	Dog	30.0
Schmidt (18).....	Dog (perfusion)	22.5
Hou (10).....	Dog	16.8-23.4
Yamakita (24).....	Rabbit	28.2
Chute and Smyth (4).....	Cat (perfusion)	9.9-15.0
<i>In vitro</i>		
Warburg (22).....	Rat cortex slice	10.7
Loebel (12).....	Rat cortex slice	11.4-13.7
Dickens et al. (5).....	Rat cortex slice	12.4
Jowett et al. (11).....	Guinea pig cortex slice	11.8
Quastel et al. (16).....	Guinea pig cortex slice	14-15
Elliott et al. (7).....	Rat gray matter homogenate	15.5
Elliott et al. (7).....	Rat whole brain homogenate	11.1

The differences within the *in vivo* group are so great as to cast doubt upon the validity of some of the figures, but whether one should prefer the largest (on the general ground that the necessary experimental abnormalities would tend to reduce cerebral metabolism) or the smallest (because of the strong probability that not all of the blood flow measured as cerebral and used in the calculation of  $Q_{O_2}$  actually went to the brain) is utterly unknown. The variations within the *in vitro* group are smaller, but the differences between these and the *in vivo* values leave doubts as to whether the consistency of the *in vitro* figures indicates that a true physiological constant is being dealt with, or whether the artificial conditions

<sup>1</sup> The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the University of Pennsylvania. Financial support was also received from the National Committee for Mental Hygiene from funds granted by the Committee on Research in Dementia Praecox founded by the Supreme Council, 33° Scottish Rite, Northern Masonic Jurisdiction, U. S. A. Permission for publication has been granted.

<sup>2</sup> The expression  $Q_{O_2}$  has come into general use for this purpose. It is defined (14) as  $\mu$ l. of  $O_2$  used per milligram of dry tissue per hour. Obviously the quantity would be the same if milliliter per gram of tissue were used instead, but it is important to note that the  $Q_{O_2}$  is referred to dry weight (which in the brain is about 20 per cent of the wet weight) and that the unit of time is specified as one hour.

of the *in vitro* experiment lead to abolition of a large proportion of the normal metabolic activity and leave only a relatively rugged moiety to be called the  $Q_{10}$ .

These questions had a certain amount of practical importance in relation to neurology and psychiatry during peace time, but the current emergency has greatly increased the need for more precise information about them. This is because the functional capacity of the human brain is definitely the limiting factor in the activities of implements of warfare such as airplanes, tanks, submarines and certain other terrestrial and aquatic vehicles, as it is also the critical factor in survival from shock, hemorrhage and anoxia due to disease, high altitude, or the effects of noxious gases. Therefore more precise information about the metabolism of the living brain *in vivo* offers some prospect of improving, not only our understanding of the vulnerability of the brain to stresses such as these, but also the procedures intended to compensate for the physiological limitations thus imposed.

*In vivo* estimations of the metabolism of the brain, as of any living tissue, involve collection and analysis of representative samples of venous and arterial blood, and measurement of the volume of blood flow corresponding with these. The latter, which was the stumbling block in the past, can be circumvented by the use of an animal (the monkey) in which the anatomical relations of the cerebral circulation are favorable for such measurements, and a measuring device that is adequately dependable and accurate. This has already been done (6). The only addition required for estimations of cerebral metabolism is provision for the collection of samples of cerebral venous and of arterial blood and this has been done in the experiments about to be described.

**METHODS.** Except for 3 spider monkeys (*ateles pentadactylus*) weighing 5-5.8 kilos, the experiments were made on Indian rhesus monkeys ranging in weight from 2.8 to 4.6 kilos. They were lightly anesthetized by intraperitoneal (intramuscular in the spider monkeys) injection of nembutal in dosage of about 20 mgm. per kilo; this was supplemented by intravenous injection of 5 mgm. (total—not per kilo) doses of pentothal sodium as required. The operative procedures were the same as in the earlier experiments (6) with only two important differences: the basilar artery was exposed through a trephined opening, the button of bone being replaced and sealed with bone wax after the artery was tied, and both internal jugular veins were exposed and their major branches ligated as far back toward the skull as possible. A flexible plastic catheter having an outside diameter of 2 mm. and a bore of 1 mm. was passed up each internal jugular as far as it would go and tied in place; the two catheters were joined by a glass Y-connection for the collection of cerebral venous blood; the veins were also ligated on the cardiac side to prevent reflux bleeding. A tracheal cannula was inserted and was connected, through a suitable cannister containing soda lime, to a counterbalanced recording spirometer having a total capacity of 500 cc., usually with a set of water valves in the circuit to humidify the inspired gas, minimize rebreathing, and facilitate the use of other gas mixtures if desired; this closed respiratory system was filled with oxygen, flushed at suitable intervals to remove exhaled nitrogen, and used to record both the total oxygen consumption and the depth and rate of the respirations. In case of respiratory failure the pulmonary gas exchange was cared for by intratracheal insufflation of oxygen through a small rubber catheter. Systemic blood pressure was measured from a femoral artery by means of a mercury manometer. Intravenous injections were made through a burette-cannula system connected with a femoral vein. Coagulation of the circulating blood was prevented by intravenous injection of a single dose of Pontamine

Fast Pink BL (100 mgm. per kilo) supplemented by 1000 unit doses of heparin at hourly intervals.

The measurement of cerebral blood flow was accomplished, as in our earlier experiments (6), by means of a bubble flow meter of about 6 ml. capacity intercalated in the stream of blood passing through both common carotid arteries, the external carotid and basilar arteries being tied. Arterial blood samples were usually collected from the internal carotid end of the flow meter, occasionally from a femoral artery. Several attempts were made to measure the basilar blood flow as in our previous experiments but these were unsuccessful because the rhesus monkeys used in the present work were smaller and less tolerant of the operative procedures than those used before, and the spider monkeys turned out to have very small basilar arteries.

The course of a complete experiment was as follows: The intravenous injection system was installed (with the aid of 5-10 mgm. doses of pentothal sodium intraperitoneally or procaine locally) so that the otherwise insufficient nembutal narcosis could be supplemented by intravenous pentothal as required. These and the subsequent operations were accomplished with all possible precautions against bleeding, a high frequency electric or a simple heated iron cautery being used liberally, and all grossly visible blood vessels being tied. The internal jugular and carotid vessels were exposed on both sides. All accessible branches of the former cephalad to the point of insertion of the catheters were tied and ligatures were passed around the external carotids on the cardiac side of the origin of any branches. The tracheal and femoral arterial cannulae were then inserted and records of blood pressure, oxygen consumption and pulmonary ventilation were begun. The internal jugular catheters (previously filled with heparin solution) were inserted and tied in place. A cannula was introduced into the other femoral artery for the collection of arterial blood. Then a pair of samples of blood (cerebral venous and arterial) was collected to provide orientation as to the cerebral A-V oxygen difference under conditions as close to normal as possible. (This was not done in all experiments.) The blood withdrawn was usually replaced by an equal volume of blood saved from the preceding monkey, or of isotonic gelatin in saline. Then the operation for exposure of the basilar artery (the most formidable part of the preparation) was carried out; in some cases the artery was tied at once, in others the ligature was passed but not tied until the carotid cannulae had been inserted, decision on this being based on the presence or absence of arterial bleeding from rupture of small branches when the ligature was passed. The anticoagulants were then injected, the external carotids tied, and installation of the carotid cannulae carried out in such a way that at least one internal carotid was always carrying blood; flow was established through both carotids before the meter was connected. The meter was filled with gelatin solution from a by-pass to the femoral venous injecting system and then blood was admitted from the carotid cannulae, displacing gelatin and carrying any air bubbles present into the femoral vein. Flow through the meter was then established by clamping the bypass to the femoral vein, and a previously patent U-connection between the pairs of cannulae, as soon as a steady state was attained. The blood samples for the control period were collected, a measurement of blood flow being made during the collection of venous blood. The remainder of the experiment was then proceeded with. Depending on circumstances, this involved collection of blood samples (with simultaneous measurement of blood flow) during spontaneous changes, following withdrawal and reinjection of varying amounts of blood, infusions of additional amounts of blood or gelatin solution or of epinephrine in gelatin (to increase cerebral blood flow), and the intravenous or intracarotid injection of convulsant doses of metrazol, picrotoxin and nikethamide, of depressant doses of pentothal, and a few other agents.

The blood samples, which measured about 5 ml. each, were collected over mercury in small tonometers, chilled at once, and analyzed for CO<sub>2</sub> and O<sub>2</sub> content within 8 hours by the manometric method; all analyses were done in duplicate and the usual precautions were taken (14).

At the end of each experiment the animal was exsanguinated and an injection of India

ink was made through the internal carotids. The distribution of the ink, in the brain and elsewhere, was checked by dissection, as was also the position of the jugular catheters. The brain was then removed and weighed anterior to the level of the basilar ligature, this factor being used in calculating blood flow and metabolism per unit weight of tissue. The results of these terminal injections confirmed those of the earlier experiments: the only extracerebral tissues injected were the eyes, eyelids and orbital tissues, with occasional small patches in the temporal and occipital musculature. The tips of the venous catheters either were just outside the skull and clearly visible from the inside, or had entered the cranial cavity and turned backward (usually) or forward into the cerebral sinus system. Collection of venous blood was made from both sides because of the fact (3) that one jugular may drain the entire cortex, the other the ventricular and choroid plexus system; a sample of blood drawn from one jugular bulb, or even from the cerebral sinuses draining into it, therefore cannot be counted upon to be truly representative of cerebral venous blood.

A total of 32 experiments were made. Of these 6 had to be discarded either because of premature death of the animal or technical imperfections such as a patent vertebral artery.

RESULTS. 1. *The "normal" metabolic rate of the brain.* The results of our 11 best experiments are summarized in table 1. These are selected on the following basis: *a*, cerebral A-V oxygen differences of the same order as those found before extensive operation and ligation of blood vessels; *b*, active cerebral functions, evidenced in brisk ocular reflexes, respiration and total oxygen consumption similar to those seen early in the experiment, and occasional spontaneous muscular movements. We believe these to be as close to the metabolic activity of the living brain *in vivo* as it is possible to get by a method involving as extensive dissection as this. The figures for  $O_2$  consumption can be converted to  $Q_{O_2}$  ( $\mu$ l. per mgm. dry tissue per hour) by multiplying them by three<sup>3</sup>.

2. *Variations in cerebral metabolism.* All of our findings are presented in figure 1 as a smoothed frequency graph showing the distribution of different levels of cerebral  $O_2$  consumption in relation to different levels of cerebral functional activity. The tendency of cerebral  $O_2$  uptake to run parallel with cerebral functional activity is quite evident. There is some overlapping but this is not surprising in view of the arbitrary basis for the classification into these three categories. The differences among the three mean values are statistically significant.

The lowest figure for cerebral  $O_2$  consumption (per 100 grams of fresh brain per minute) was 0.39 ml. in a moribund animal without any signs of cerebral activity. The highest was 6.5 ml. during a picrotoxin convulsion (table 4, expt. 27). The lowest compatible with subsequent recovery of cerebral activity was 1.85 ml. following hemorrhage (table 2, expt. 26). The "physiological" range of cerebral  $O_2$  uptake under these experimental conditions therefore was from nearly double to about half the resting "normal" value. (See table 6, p. 44.)

3. *Effects of intentional decrease or increase in cerebral blood flow.* (a) *Decrease.*

<sup>3</sup> This is derived as follows, taking the average  $O_2$  consumption as an example: (1) Divide by 100 to reduce to milliliter per gram (or  $\mu$ l. per mgm.) per minute (= 0.087). (2) Multiply by 5 to convert to dry weight (= 0.185). (3) Multiply by 60 to convert to  $O_2$  uptake per hour (= 11.1). Dividing by 100 and then multiplying by 300 gives the same result as simply multiplying by 3.

This was brought about by removing appropriate amounts of blood. As usual in bleeding experiments, subsequent reinjection of the blood in some cases brought about complete and (for our purposes) permanent restoration of circulation, respiration and ocular reflexes, while in others the improvement was only partial and temporary. Cerebral blood flow was invariably decreased by the

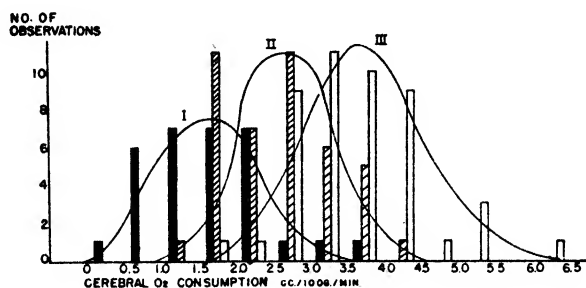


Fig. 1. Relation of cerebral  $O_2$  consumption to cerebral functional activity, arranged in three groups: I spontaneous breathing, ocular reflexes and all other signs of cerebral activity entirely absent (black rectangles indicate observations in this group); II spontaneous breathing of some type present but ocular reflexes and muscular movements absent (indicated by cross-hatched rectangles); III breathing normal, ocular reflexes and occasional movements of extremities present (indicated by open rectangles). The mean values and their standard errors are  $1.7 \pm 0.15$ ,  $2.6 \pm 0.13$  and  $3.7 \pm 0.16$  for groups I, II, and III respectively.

TABLE 1

EXPT. NO.	GENERAL CONDITION	BLOOD PRES- SURE	CEREBRAL				O <sub>2</sub> CONSUMED		CERE- BRAL R.Q.	WEIGHT		
			A-V diff.		Blood flow	O <sub>2</sub> uptake	Total	% by brain		Animal	Brain	
			O <sub>2</sub>	CO <sub>2</sub>								
			vols. %	ml./100g./min.								ml./ min.
5	Good—light anesthesia	135	7.0	6.0	53.4	3.7			0.85	2.8	90	
9	Good—deep anesthesia	84	6.6	6.9	38.0	2.5			1.05	3.2	90	
13	Good—light anesthesia	114	8.7	7.3	33.5	2.9	29	9.0	0.84	3.5	88	
14	Excellent—active movements	100	10.1	10.2	33.4	3.4	30	11.7	1.01	3.5	114	
22	Excellent—active movements	134	7.8	8.0	41.0	3.1	40	6.9	1.02	4.1	89	
24	Excellent—reflexes very active	124	6.0	6.0	73.6	4.4	31	12.1	1.00	3.7	85	
25	Excellent—reflexes very active	150	9.7	8.2	46.6	4.5	41	8.8	0.85	4.1	80	
26	Excellent—reflexes very active	116	8.2	7.6	40.4	3.3	31	8.7	0.92	3.3	82	
27	Excellent—reflexes very active	102	8.8	10.7	49.7	4.4	35	9.4	1.22	3.0	75	
30	Excellent—reflexes very active	110	7.8	7.5	53.0	4.1	37	12.3	0.96	5.8	105	
31	Excellent—reflexes very active	100	8.2	8.1	53.7	4.4	48	10.0	0.99	5.3	108	
Average .....			115	8.0	8.0	46.9	3.7	36	10.0	1.00	3.8	91

hemorrhage and increased by the subsequent transfusion; the A-V oxygen difference underwent changes exactly the opposite of these. In the cases of complete recovery after transfusion cerebral  $O_2$  uptake came back to or above its control level while in the others it did not. Representative examples of these responses are shown in table 2. The data suggest an inverse rather than a direct relationship between the intensity of cerebral metabolism and the ability of the

brain to withstand the anoxia produced by hemorrhage, but the observations are too few to warrant any categorical statements at the present time.

(b) *Increase.* This was brought about by intravenous infusion of epinephrine (1:20,000 or 1:100,000 in isotonic gelatin) in two spider monkeys in excellent condition, also in one spider and four rhesus animals as a restorative measure after breathing had ceased and reflexes had disappeared. The effects on cerebral blood flow and A-V oxygen difference were uniformly the reverse of those produced by hemorrhage. Cerebral O<sub>2</sub> uptake, however, behaved differently according to the state of cerebral activity before the epinephrine was given. If the

TABLE 2

EXPT. NO.	EXPERIMENTAL CONDITIONS	BLOOD PRESSURE	O <sub>2</sub> CONTENT		CO <sub>2</sub> CONTENT		CEREBRAL			O <sub>2</sub> CONSUMED		CONDITION OF ANIMAL	CORNEAL REFLEX
			Art.	Vein	Art.	Vein	AV-O <sub>2</sub>	Blood flow	O <sub>2</sub> uptake	Total	% by brain		
		mm. Hg	vols. %		vols. %		vols. %	ml./100 g./min.		ml./min.			
26	Control	116	14.4	6.1	45.6	53.2	8.2	40.4	3.30	31	8.7	Excellent	++
	Bleeding 10 ml.	88	14.5	5.3	45.5	55.2	9.3	33.9	3.14	28	9.2	V. good	++
	Bleeding 10 ml.	70	14.9	3.3	43.9	56.3	11.7	27.9	3.26	28	9.6	V. good	++
	Bleeding 10 ml.	58	14.8	0.8	41.2	57.4	14.0	20.2	2.83	25	9.3	Fair	±
	Bleeding 10 ml.	44	14.7	0.6	35.8	55.8	14.1	13.2	1.85	21	7.2	Bad	0
	Blood returned	114	11.6	3.4	46.0	53.7	8.1	46.0	3.72	34	9.0	Good	+
	15 min. later	110	12.9	3.4	47.4	57.3	9.5	41.4	3.94	35	9.2	Good	+
24	Control	124	11.6	5.6	50.9	56.9	6.0	73.6	4.43	31	12.2	Excellent	+++
	Bleeding 10 ml.	80	12.7	3.4	53.5	61.3	9.3	39.6	3.69	28	11.1	Fair	+
	Bleeding 6 ml.	64	12.5	2.2	54.6	64.4	10.3	30.2	3.12	27.5	9.8	Bad	0
	Blood returned	105	12.2	3.2	51.9	60.3	9.0	32.2	2.92	32	7.8	Bad	0
	Gelatin 10 cc.	138	11.4	5.1	51.5	67.5	6.3	56.5	3.55	34	8.8	Good	++
30	Control	110	12.5	4.7	52.1	59.6	7.8	53.0	4.13	35	12.3	Excellent	++++
	Bleeding 75 ml.	56	10.3	1.5	49.7	59.6	8.8	32.9	3.04	27	11.1	Good	++
	Bleeding 35 ml.	40	9.4	0.9	45.7	54.8	8.5	24.6	2.09	12	18.3	Bad	±
	Blood returned	100	10.0	5.3	45.1	49.1	4.7	62.2	2.93	35	8.9	Good	++
25	Control	150	14.6	4.9	45.9	54.1	9.7	46.6	4.52	41	8.8	Excellent	+++
	Bleeding 10 ml.	100	15.2	2.0	46.7	58.3	13.2	29.5	3.90	32	9.7	Fair	+
	3 min. later	96	13.7	1.3	51.6	59.6	12.4	28.0	3.48	30	9.0	Bad	0
	Transfused 25 ml.	114	13.2	3.3	57.5	65.4	9.9	31.2	3.10	art.	resp.	Bad	0
	30 min. later	56	8.1	3.2	79.6	83.0	4.9	11.2	0.56	art.	resp.	Moribund	0

latter was already optimal (normal breathing, brisk ocular reflexes) the O<sub>2</sub> consumed by the brain underwent a decrease while if reflexes had disappeared and respiratory gasps or failure and low blood pressure gave indication of deterioration of central nervous functions, cerebral O<sub>2</sub> consumption was increased by the epinephrine. Examples of these responses are shown in table 3.

4. *The effects of convulsant and narcotic drugs.* (a) *Convulsants.* Metrazol, picrotoxin, nikethamide (coramine) and benzedrine were used (table 4).

Metrazol, given intra-arterially or intravenously in about 100 mgm. dosage to 4 responsive animals, led almost immediately to characteristic clonic convulsions involving the face, trunk and limbs. At the same time cerebral blood flow and O<sub>2</sub> uptake were consistently and considerably increased while the A-V oxygen

difference showed relatively slight and inconstant changes. These effects were brief and were typically followed by a prolonged diminution in cerebral functional activity, blood flow and O<sub>2</sub> uptake, with no consistent change in the A-V difference. Given in the same or larger dosage to animals showing no signs of central nervous activity, metrazol was without conspicuous effect on cerebral activity, blood flow, or O<sub>2</sub> uptake. The latter effects therefore depend on the change in functional activity, not on a direct action by the drug on the cerebral blood vessels.

Picrotoxin was given in 3 and 7 mgm. total dosage to 2 animals. It had more marked and more prolonged effects than metrazol. In one experiment (no. 28)

TABLE 3

EXPT. NO.	EXPERIMENTAL CONDITIONS	BLOOD PRES- SURE	O <sub>2</sub> CONTENT		CO <sub>2</sub> CONTENT		CEREBRAL			O <sub>2</sub> CONSUMED		CONDITION OF ANIMAL	COR- NEAL REFLEX	BRAIN WEIGHT
			Art.	Vein	Art.	Vein	A-VO <sub>2</sub>	Blood flow	O <sub>2</sub> up- take	Total	% by brain			
		<i>mm. Hg</i>	<i>vols. %</i>		<i>vols. %</i>		<i>vols. %</i>	<i>ml./100 g./min.</i>		<i>ml./ min.</i>				<i>grams</i>
30	Control (nat. resp.)	110	12.5	4.7	52.1	59.6	7.8	53.0	4.13	35	12.3	Excellent	++++	105
	Epineph. infusion	125	10.7	4.6	51.8	58.0	6.1	62.3	3.80	33	12.2	Excellent	++++	
31	Control (nat. resp.)	96	15.4	6.1	46.6	55.0	9.3	45.9	4.26	53	8.7	Excellent	++++	108
	Epineph. infusion	145	13.0	8.4	49.2	52.8	4.6	70.8	3.53	29	13.1	Excellent	++++	
	After epineph.	79	10.6	2.6	45.6	52.3	8.0	51.0	4.08	75	5.9	Excellent	++++	
32	Control (art. resp.)	36	9.6	0.8	67.8	74.6	8.8	21.0	1.85			Bad	0	95
	Epineph. infusion	112	8.5	4.9	66.6	69.0	3.6	85.6	3.08			Fair	+	
8	Control (art. resp.)	32	11.7	2.0	44.3	54.7	9.7	18.9	1.83			Bad	0	95
	Epineph. infusion	94	11.5	6.5	43.9	48.3	5.0	41.5	1.96			Bad	0	
	Epineph. continued	88	9.9	5.7	42.1	46.3	4.2	44.0	1.85			Bad	0	
15	Control (art. resp.)	64	14.2	4.0	81.1	89.3	10.2	17.7	1.80			Bad	0	109
	Epineph. infusion	154	12.5	7.1	80.3	87.3	5.4	44.7	2.40			Bad	0	
	After epineph.	46	8.2	1.8	83.7	90.4	6.4	9.6	0.61			Moribund	0	
12	Control (art. resp.)	77	13.1	8.5	67.7	79.9	4.6	22.7	1.05			Bad	0	90
	Epineph. infusion	164	11.1	5.7	67.8	72.1	5.4	22.4	1.21			Bad	0	
	After epineph.	54	6.9	2.8	52.8	61.1	4.1	10.6	0.43			Moribund	0	

spontaneous breathing was restored and an improvement in blood pressure (probably vasomotor in origin) occurred after the administration of this drug. The increase in cerebral metabolism was due to increase in both A-V difference and blood flow but the preponderance differed in the two animals. The subsequent depressant phase was as evident as with metrazol.

Nikethamide was injected into the carotid stream in 50 to 500 mgm. dosage in 3 monkeys at varying stages of central nervous activity but in only one were there any signs of stimulation of the central nervous system by the drug. Its effects on blood pressure, respiration and cerebral blood flow were peculiar. The first effects after the injection were cessation of respiration, fall in blood pressure, and decrease (amounting in one case to complete standstill for about 20 sec.) in cerebral blood flow. Blood pressure then rose again, spontaneous breathing



TABLE 4

EXPT. NO.	EXPERIMENTAL CONDITIONS	BLOOD PRESSURE	O <sub>2</sub> CONTENT		CO <sub>2</sub> CONTENT		CEREBRAL			O <sub>2</sub> CONSUMED		CONDITION OF ANIMAL	COR-NEAL REFLEX	BRAIN WEIGHT
			Art.	Vein	Art.	Vein	A-VO <sub>2</sub>	Blood flow	O <sub>2</sub> uptake	Total	% by brain			
		mm. Hg	vols. %	%	vols. %	%	vols. %	ml./100g./min.	ml./min.					grams
13	Control (nat. resp.)	114	16.7	8.1	40.9	48.2	8.6	33.5	2.90	29	9	V. good	++	88
	Metrazol 100 mg. i.a.	96	15.4	4.5	31.5	49.2	10.9	47.5	5.17	34	13.8	Convuls.	++	
	Pentothal 5 mg. i.a.	74	16.5	9.8	35.3	36.6	6.7	38.0	2.89	31	9.3	Depressed	0	
	8 min. later	56	14.1	3.0	30.0	42.7	11.1	17.1	1.89	29	5.9	Better	+	
	Transfusion	83	13.2	3.0	34.4	44.1	10.2	31.1	3.16	35	8.0	V. good	++	
14	Control (nat. resp.)	100	13.5	3.5	39.6	49.7	10.0	33.4	3.36	32.6	11.6	Excellent	++++	114
	Pentothal 5 mg. i.a.	100	13.9	2.6	42.9	52.6	11.3	19.0	2.15	26	8.9	Depressed	0	
	Metrazol 100 mg. i.a.	80	12.7	0.6	37.3	46.9	12.1	28.8	3.50	25	16	Convuls.	++	
	15 min. later	64	14.1	2.3	45.7	54.7	11.8	17.3	2.04	30	7.7	Depressed	0	
	30 min. after met.	110	11.7	2.4	43.3	53.1	9.3	31.4	2.92	36	9.2	V. good	++	
10	Control (nat. resp.)	100	12.9	5.0	58.3	68.3	7.9	29.2	2.30			Poor	0	85
	Metrazol 100 mg. i.a.	136	11.1	4.2	56.7	69.4	6.9	59.8	4.13			Convuls.	++	
	15 min. later	100	5.7	1.6	66.3	71.0	4.1	20.6	0.85			Bad	0	
18	Control (nat. resp.)	94	15.4	3.4	14.4	27.6	12.0	22.6	2.71	29.4	10.9	Fair	0	102
	Metrazol 100 mg. i.a.	124	14.6	2.9	15.7	27.0	11.7	30.1	3.53	33.3	9.3	Convuls.	++	
8	Control (art. resp.)	88	9.9	5.7	42.1	46.3	4.2	44.0	1.85			Bad	0	95
	Metrazol 100 mg. i.a.	80	10.1	7.1	39.8	44.1	3.0	65.7	1.97			Sl. convul.	++	
32	Control (art. resp.)	112	8.5	4.9	66.6	69.0	3.6	85.6	3.08			Bad	0	95
	Metrazol 200 mg. i.v.	80	5.3	0.6	61.9	68.4	4.7	62.2	2.92			No convul.	0	
	Coramine 500 mg. i.v.	80	6.3	0.8	36.4	51.7	5.5	66.4	3.65			Convuls.	++	
	17 min. later	40	5.8	0.3	40.4	52.8	5.5	27.4	1.51			Bad	0	
	12 min. later	34	5.7	0.3	45.9	53.3	5.4	24.6	1.32			Moribund	0	
22	Control (nat. resp.)	128	8.5	2.4	62.0	67.6	6.1	32.1	1.95	42	4.0	Poor	0	
	Coramine 50 mg. i.a.	70	8.8	2.5	65.4		6.3	26.6	1.67	40	3.8	No convul.	0	
	9 min. later	122	8.8	2.6	59.7	67.0	6.2	29.3	1.82	37	4.3	Poor	0	
	Pentothal 5 mg. i.a.	52	9.1	2.7	71.3	74.8	6.4	13.5	0.87			Bad	0	
24	Control (nat. resp.)	106	11.1	4.1	51.5	60.0	7.1	54.2	3.85	36	6.2	Good	+	85
	Coramine 50 mg. i.a.	130	9.7	4.5	49.9	56.8	5.2	64.4	3.24	41	6.8	No convul.	+	
	4 min. later	106	8.4	0.7	55.6	60.5	7.7	28.7	2.21	31	6.1	Poor	0	
27	Control (nat. resp.)	102	13.0	4.2	40.2	50.9	8.8	49.7	4.37	35	9.4	Excellent	++++	75
	Picrotoxin 3 mg. i.a.	116	12.5	3.6	41.7	50.7	8.9	57.0	5.06	40	9.5	Convuls.	++++	
	19 min. later	70	12.7	3.7	51.7	62.8	9.0	41.0	3.68			Poor	0	
	8 min. later	102	12.8	2.0	53.8	65.0	10.8	60.1	6.50			Twitching	++	
	8 min. later	80	12.5	2.1	49.4	62.5	10.4	51.5	5.35			Convuls.	++	
	42 min. later	124	11.8	2.0	44.8	51.8	9.8	48.6	4.77	36	10	Excellent	++++	
	Pentothal 2.5 mg. i.a.	116	11.4	3.1	46.2	54.3	8.3	45.9	3.81	38	7.6	Depressed	±	
	15 min. later	124	10.5	2.2	46.5	52.5	8.7	50.3	4.37	38	8.7	Excellent	++++	
28	Control (art. resp.)	83	14.5	7.5	68.4	74.9	7.0	33.8	2.36			Bad	0	88
	Picrotoxin 7 mg. i.a.	104	14.2	2.0	70.2	84.1	12.2	34.6	4.22	36	10.2	Good	+	
	25 min. later	102	14.1	1.9	65.5	78.4	12.2	29.2	3.57	36	8.6	Good	+	
	Benzedrine 1 mg. i.a.	106	12.7	3.1	65.0	75.8	9.6	27.0	2.59	36	6.4	Poor	0	
	18 min. later	106	11.8	2.6	67.1	74.9	9.2	30.5	2.80	52	4.8	Fair	0	
2	Control (art. resp.)	80	9.5	3.5	34.4	38.3	6.0	25.2	1.51			Bad	0	95
	Benzedrine 5 mg. i.v.	94	10.5	3.3	35.4	40.1	7.2	29.0	2.1			Bad	0	
	5 min. later	86	6.7	2.0	36.5	40.7	4.7	25.4	1.2			Bad	0	
5	Control (art. resp.)	64	12.7	4.6	38.4	47.5	8.1	36.4	2.95			Bad	0	90
	Benzed. 5 mg. i.v.	70	11.0	1.7	36.9	47.1	9.3	20.8	1.94			Bad	0	

returned, cerebral blood flow came back to or above its control level, and the convulsant effects of the drug now appeared for the first time.

Benzedrine was given in a number of experiments to test its effects on cerebral blood flow but only in 3 of these was there a sufficiently steady state to justify the collection of blood samples. Cerebral metabolism was somewhat decreased in 2 of the cases, increased in the third. Cerebral blood flow behaved like cerebral metabolism. Blood pressure rose in all 3 but there were no manifestations of stimulation of the central nervous system.

Representative examples of these results are shown in table 4. Cerebral  $O_2$  uptake and blood flow were invariably increased during the convulsant period by metrazol, nikethamide and picrotoxin, and this therefore appears to be the characteristic pattern of the action of such drugs. The behavior of the A-V oxygen difference was so inconstant as to indicate that factors at present unidentified are involved here. The absence of effect on either metabolism or blood flow when no convulsions were seen indicates that the increase in blood flow is related to the convulsant action and is not simply due to dilatation of cerebral vessels by the drug itself. The convulsant period was invariably followed by depression of respiration, ocular reflexes, cerebral blood flow, and cerebral  $O_2$  uptake, the latter reaching approximately the level attained by a distinctly narcotic dose of pentothal (table 4). The time relations of these various events differed considerably among the three convulsants. With metrazol the maximum effects on cerebral activity, blood flow and metabolism were seen almost immediately after the injection and the stimulant phase lasted only a minute or two. With picrotoxin the effects came on more gradually but were much more prolonged; in one case (expt. 27) the initial stimulant phase was followed by a period of depression and this by a return of the convulsant effects of the original dose of the drug, indicating that the initial stimulant action was terminated by deterioration in the ability of the brain to react, not by excretion or detoxification of the drug. With nikethamide there was an initial depressant action on cerebral functions and blood flow that was lacking with the other two. The stimulant phase came on after this (if at all) and was accompanied by the same type of increase in cerebral blood flow and metabolism that was seen with the others. The subsequent depression of cerebral functional activity, metabolism and blood flow seemed to depend more on the vigor of the convulsant response than on the drug used and was of about the same order of intensity and duration with all 3 agents.

Benzedrine, in the dosage employed and under the existing experimental conditions, gave no sign of an ability to stimulate the brain in a manner comparable with the convulsants. The effects obtained by it could be ascribed to its sympathomimetic actions associated with a mild constriction of cerebral blood vessels; the latter action would be a unique and unfavorable concomitant of stimulation of brain cells, but it is possible that if such stimulation occurred the relatively mild cerebral constriction would give way to vasodilatation. On the other hand, if stimulation did not occur the cerebral vasoconstrictor action would be distinctly disadvantageous.

(b) *Depressants*. Pentothal sodium is the only agent with which enough ob-

servations were made to warrant definite statements. It was chosen because of the brevity and complete reversibility of its effects. When given into the carotid stream in suitable dosage (2-5 mgm. total) to animals with active respiration and reflexes, it caused distinct depression of rate and depth of breathing and disappearance of the ocular reflexes, but artificial pulmonary ventilation usually was not required and systemic blood pressure was not significantly altered. At the height of this effect cerebral  $O_2$  uptake and blood flow were both reduced consistently and considerably while the A-V oxygen difference showed no consistent change. Pentothal was also given to counteract the convulsant effect of metrazol; the effects on cerebral blood flow, A-V difference and metabolism were of the same type as those just described though the true effect of the depressant drug is not as clear because of the spontaneous tendency toward a temporary depressant phase in these functions following the convulsant period. Examples of these effects are shown in table 4.

5. *Correlations among cerebral blood flow, A-V oxygen difference and oxygen uptake.* (a) *Between cerebral blood flow and A-V oxygen difference.* Our data bearing on this are shown in figure 2. In this (as well as in figs. 3, 4 and 5) each point represents one set of blood samples with its corresponding observations and all of the data are included without selection. Although in a given experiment there may be good correlation between these two factors within certain limits (tables 2 and 3), when all the findings in all the experiments are viewed together this relationship has a correlation coefficient of  $-0.18$ , which is the poorest of the three possible ones among these three variables.

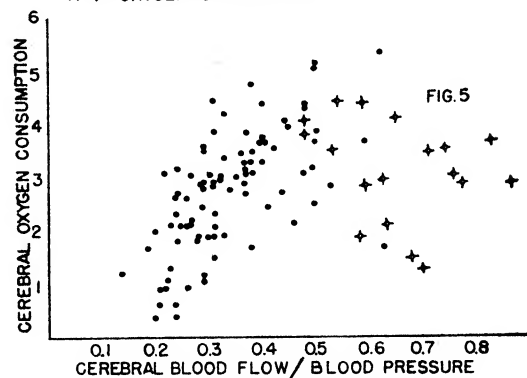
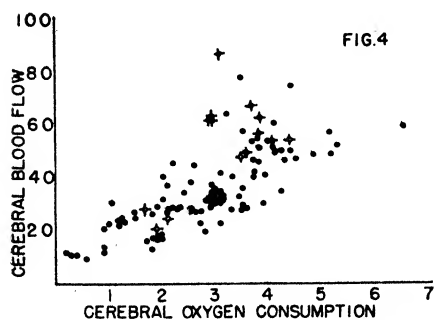
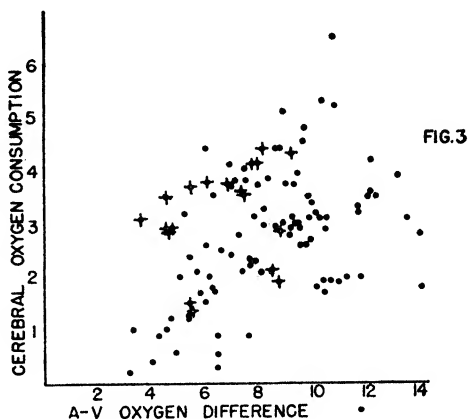
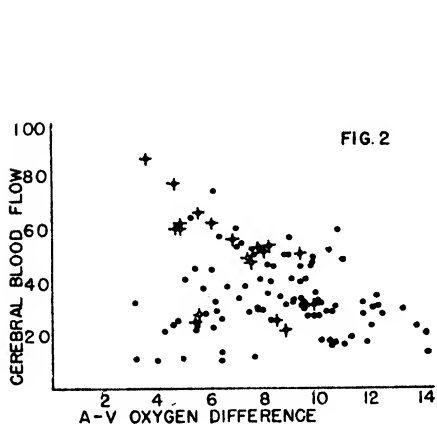
(b) *Between cerebral oxygen consumption and cerebral A-V oxygen difference.* Our findings on this are plotted in figure 3. The correlation coefficient here is  $0.54$ , which is considerably better than (a).

(c) *Between cerebral oxygen consumption and cerebral blood flow* (fig. 4). The coefficient of correlation here is  $0.67$ , which is the best of the three. This suggests that the cerebral circulation automatically adjusts itself to the existing metabolic requirements of the brain—a suggestion that is borne out by figure 5, which shows the relationship between cerebral metabolic activity and cerebral blood flow corrected for differences in blood pressure, so as to disclose changes in cerebral vascular tone. The coefficient of correlation for the findings in rhesus monkeys is  $0.59$ , which indicates a significant relationship. The data from spider monkeys fall completely out of line, the blood flow per unit of tissue and blood pressure tending to be considerably greater than in the rhesus monkeys. This is already evident in table 1, in which numbers 30 and 31 were ateles and the others were rhesus. A species difference is strongly suggested but the data are insufficient to establish a separate set of correlations. No corresponding differences are evident in the other relationships (figs. 2, 3 and 4).

6. *Other observations.* (a) *The effects of insulin.* Only one of three experiments with this drug was satisfactory. The results are shown in table 5. The absence of any striking change in cerebral  $O_2$  uptake is noteworthy. The animal's condition was excellent and spontaneous muscular movements, which were present before insulin, increased during the hypoglycemic period. The decrease in  $O_2$

uptake following glucose injection was probably of the same nature as that seen with epinephrine in "normal" animals (table 3). Unfortunately this animal showed no trace of hypoglycemic shock in spite of a total of 20 units of insulin and these observations therefore apply only to the stage of moderate ("therapeutic") insulin action.

(b) *The effects of changes in arterial  $pO_2$  and  $pCO_2$ .* Observations along these lines were incidental and were usually made after the planned experiment on



Figs. 2, 3, 4 and 5. The values for cerebral blood flow and cerebral  $O_2$  consumption are plotted in terms of milliliters per 100 grams wet brain weight per minute, those for A-V oxygen difference in volumes per cent. In figure 5 the observed blood flow values have all been divided by the blood pressure at the time of measurement. The observations on spider monkeys are shown as stars; all the others were made on rhesus animals.

cerebral metabolism had been made. The results confirmed those of our earlier work on monkeys (6) in all essential respects: inhalation of 10 per cent  $CO_2$  in  $O_2$  had little or no effect on cerebral blood flow unless blood pressure underwent a change, which the flow followed apparently passively. Anoxemia was associated with an increase in flow provided that cerebral activity was present, but not otherwise. Induced hyperventilation with  $O_2$  did not decrease the blood flow unless blood pressure fell. The extent of the changes in arterial gas tensions was not determined.

(c) *The effects of breathing against pressure.* These observations, like the preceding, were incidental and were made on animals that were still in good condition after the planned metabolism experiment had been made. The pressure was hydrostatic and was applied to the outlet of the expiratory valve. It was therefore continuous throughout the respiratory cycle. The inspired gas was 100 per cent  $O_2$ . Satisfactory tests were made on 5 rhesus monkeys. The results are adequately summarized by stating that cerebral blood flow did not change until the blood pressure did, when both changed in the same direction. Pressures up to 3 inches of water only slowed breathing somewhat without affecting blood pres-

TABLE 5

EXPT. NO.	EXPERIMENTAL CONDITIONS	B-P	O <sub>2</sub> CON-TENT		CO <sub>2</sub> CON-TENT		CEREBRAL			O <sub>2</sub> CON-SUMED		BLOOD SUGAR	CONDI-TION	C.R.	WT. OF BRAIN
			Art.	Vein	Art.	Vein	A-VO <sub>2</sub>	Blood flow	O <sub>2</sub> up-take	Total	% by brain				
			vols. %		vols. %		vols. %	ml./100g./min.		ml./min.		mgm./%			
31	Control	79	10.6	2.6	45.6	52.3	8.0	51.0	4.08	75	5.9	159	Excel.	++++	108
	10' after insulin-20 u	66	9.9	2.4	46.4	54.2	7.5	47.2	3.54	70	5.0	98	Excel.	++++	
	1 hr. after insulin	66	9.1	1.7	47.2	54.5	7.4	49.3	3.64	75	4.9	41	Excel.	++++	
	After glucose-5 g.	69	6.0	1.2	49.3	52.7	4.8	60.3	2.89	76	3.8	197	Excel.	++++	

TABLE 6

	FINDINGS IN MONKEYS' BRAINS ML./100 G./MIN.		QO <sub>2</sub>	CORRESPONDING VALUE FOR 1400 G. HUMAN BRAIN (ML./MIN.)	
	O <sub>2</sub> uptake	Blood flow		O <sub>2</sub> uptake	Blood flow
"Normal" mean.....	3.7	47	11.1	52	660
"Normal" minimum.....	2.5	33	7.5	35	460
"Normal" maximum.....	4.5	74	13.5	63	1040
Highest of all (picrotoxin convulsion) ..	6.5	91	19.5	91	1275
Lowest with recovery (hemorrhage) ....	1.85	13.2	5.6	26	185
Lowest of all (moribund).....	0.39	5	1.17	6	70

sure or cerebral blood flow but at 4 inches respiratory difficulty and fall in blood pressure were seen. None of the animals were able to breathe against 8 inches: the chest assumed the inspiratory position and the expiratory muscles apparently were not strong enough to expel gas against this pressure; blood pressure fell sharply and cerebral blood flow did likewise.

These findings indicate that the rise in systemic venous pressure associated with high intrapulmonary pressure does not lead to diminution in arterial inflow to the brain, over the range of pressure that can be borne by the respiratory and cardiovascular apparatus.

(d) *The effect of minimal movement of blood in the cerebral circulation.* Because of their possible bearing on the problem of resuscitation we wish to record here a few observations which were made possible by the unique conditions of these

experiments. In a number of cases the animal was still in good condition (active reflexes, normal type of breathing) when terminal exsanguination was begun. The bleeding was rapidly followed by gasping respiration, then by complete failure of respiration and circulation. We discovered accidentally in one such experiment that if blood was sucked out of the venous catheters into a syringe, respiratory activity could be restored and maintained for some time, and this was confirmed in subsequent similar experiments. Apparently movement of blood through the cerebral vessels led to distinct improvement in the state of the respiratory center even though the pressure in the vessels was zero (atmospheric) or below. This course of events was seen only in animals previously in good condition, with respiratory failure resulting from rapid exsanguination, and with a minimum lapse of time between the failure and the withdrawal of venous blood—a situation having its counterpart in ventricular fibrillation associated with electric shock. If the two are really comparable, the prompt application of manual artificial respiration should help to prevent total and permanent failure of the respiratory center in cases of the latter type.

(e) *The fraction of the total oxygen uptake accounted for by the brain.* In the 9 “normal” animals in which pertinent data were obtained, the average for this was almost exactly 10 per cent (table 1). The changes that took place under changing conditions are shown in the subsequent tables. It should be recalled that most of the injections of convulsants and all of the injections of pentothal were made intra-arterially so that only the brain was exposed to an effective concentration of these drugs.

(f) *The partition of the total carotid flow between the internal and external carotids.* This was studied in one spider monkey in which the basilar artery was ligated and the flow started through the meter before the external carotids were tied. The flow was 52 ml. per minute at a blood pressure of 78 mm. Hg. After the external carotids were tied the flow was 37 ml. per minute at a pressure of 80 mm. In this animal about 70 per cent of the total common carotid stream was therefore carried by the internal carotids.

DISCUSSION. The importance of the four main physiological variables dealt with in these experiments—cerebral functional activity, cerebral metabolic activity, cerebral blood flow and cerebral arteriovenous oxygen difference—is attested by the existence of a large mass of literature dealing with each. The mutual relationships among them have been the basis for considerable speculation, but as far as we are aware this is the first time that direct experimental evidence bearing upon these relationships has been obtained under conditions approximating the normal. While we cannot guarantee that these findings are applicable to animals other than the monkey, or even to that animal in the intact, unanesthetized state, we believe that they deserve full discussion because they afford a glimpse of these interrelationships under conditions which, although they cannot be called entirely normal, certainly were not incompatible with cerebral functional activity. To facilitate this discussion we have summarized some of the most important findings in table 6.

*The normal cerebral  $Q_{O_2}$ .* In view of the depression of cerebral  $O_2$  uptake by

narcosis (table 4) it seems proper to regard the highest of our "normal" values as our closest approximation to the situation in the intact, unanesthetized monkey. This (4.5 ml. per 100 grams of wet brain per min.) gives a  $Q_{O_2}$  of 13.5, which comes directly in the midst of the findings for slices of guinea pig or rat cerebral cortex *in vitro* (p. 33 above). The natural conclusion would be that the *in vitro* technic gives values that closely approximate the  $O_2$  uptake by the living mammalian brain *in vivo*. Before this conclusion can be accepted, however, two facts must be taken into consideration. One is that narcosis was present throughout the present experiments; even the highest "normal"  $Q_{O_2}$  should have been somewhat depressed by this. The other is that these *in vivo* values are for the entire brain whereas the *in vitro* ones which they most closely resemble are for cortex alone. If the uptake of the cortex in our monkeys was 2 to 4 times that of subcortical areas, as *in vitro* studies have indicated it to be in other animals (15), it follows either that the metabolic rate of the monkey's cortex must have been much higher than our figure, or that practically all of the measured blood flow and  $O_2$  uptake were accounted for by the cortex. The latter seems improbable in view of the active state of the ocular reflexes and of the respiratory and vasomotor centers. The former alternative therefore is preferable, but in that case the agreement between our  $Q_{O_2}$  and that of cortical slices probably was purely fortuitous.

It is noteworthy that even the highest of our  $Q_{O_2}$  values is considerably below any of those previously reported for the mammalian brain *in vivo* (p. 33). This discrepancy almost certainly is due to overestimation of the blood flow factor in the earlier experiments because of escape of blood through anastomoses with the extracranial circulation. This subject is considered in detail elsewhere (20) and needs no further discussion here. The "physiological" range of the  $Q_{O_2}$  (i.e., the highest attainable with a convulsant and the lowest compatible with complete recovery) turns out to be from nearly double to about half the resting "normal" value (p. 36 above). Lower values than this were encountered but since neither cerebral functions nor cerebral  $O_2$  uptake could then be restored such levels probably correspond with irreversible (pathological) changes in nerve cell function.

*The magnitude of cerebral blood flow.* Table 6 gives a fair representation of the range of cerebral blood flow in these animals under conditions compatible with cerebral activity, as well as the corresponding values for an adult human brain of average size. It is noteworthy that the mean and maximum blood flow values encountered in these monkeys were distinctly below the corresponding figures (60 and 113 ml. per 100 grams per min. respectively) in our earlier work (6). Part of the difference may be due to the routine use in these experiments of inhalation of 100 per cent oxygen, which tends to reduce cerebral blood flow in the monkey (6). Part may be attributable to the fact that the monkeys used in the present study were on the whole smaller and less resistant to the experimental procedures, perhaps because of longer captivity. We have not succeeded in a number of recent attempts at checking the accuracy of our earlier correction factor of 70 per cent for the reduction in cerebral blood flow upon closure of the basilar artery (6). The basilar artery of the spider monkey is so much smaller

than that of the rhesus that the same correction certainly should not be applied to both. The figures presented throughout this paper are those actually obtained and are uncorrected.

*The significance of changes in the cerebral A-V oxygen difference.* We have pointed out in section 5 that although in a given animal and within certain limits a change in the A-V difference can be correlated with a simultaneous change in either cerebral blood flow or cerebral metabolism, there is poor correlation between the A-V difference and either of these factors when the data are viewed together. The best of the three possible correlations among these variables is that between cerebral metabolism and cerebral blood flow, which, as already pointed out (p. 42), strongly suggests an effective adjustment of cerebral blood flow to the momentary metabolic requirements of the brain. An alternative explanation would be that cerebral blood flow in some manner determines cerebral metabolism. While this undoubtedly is true when a subnormal flow has become the limiting factor on cerebral metabolism, it does not appear to hold when the flow is increased above the optimal level (table 3) or reduced only enough to permit adequate compensation by increasing the A-V difference (table 2). We therefore prefer the former interpretation, which is in agreement with all our experimental findings. But if there is a completely effective mechanism for automatically regulating the blood supply of the brain in accordance with its metabolic requirements, it follows that the A-V difference of oxygen (or anything else) should be kept constant, by suitable changes in cerebral blood flow, in the face of considerable changes in cerebral metabolic activity. Any changes in the A-V difference would signify that the mechanism had not functioned perfectly but there would be no clue as to whether the primary factor was a change in blood flow or in metabolism. Our results with primary changes in blood flow (tables 2 and 3) and metabolism (table 4) show the impossibility of drawing valid deductions of this character from changes in the A-V difference alone. This question is considered in greater detail elsewhere (20).

*The relation of cerebral functional activity to cerebral metabolism.* One of the most striking and consistent findings in these experiments is the direct relation between cerebral functional activity (manifested in respiratory and vasomotor control, ocular reflexes, muscular movements) and cerebral  $O_2$  consumption. Our findings therefore confirm those already obtained in other animals with other methods (1) (18), and justify the addition of the mammalian brain to the list of tissues in which an increase in functional activity has been proved to be associated with an increase in its call for oxygen (2). The causal nature of this relationship is not disclosed by our experiments but it seems most probable that each may be the cause or each the effect, depending on circumstances. When the primary factor is reduction in the supply of  $O_2$  (as in our hemorrhage experiments, but presumably also in any other type of cerebral anoxia) the decrease in functional activity must be the result of the anoxic interference with cerebral cellular activity. Under such circumstances restoration of the  $O_2$  supply to the brain will lead to return of functional activity and  $O_2$  uptake, provided that the anoxic derangement has not progressed far enough to be partly or completely irrever-



sible. According to our results with hemorrhage the degree of reduction in cerebral  $O_2$  uptake that can be recovered from promptly and completely is about half the resting "normal".

These statements imply that reduction in the cerebral  $O_2$  supply will necessarily lead to a diminution, and increase of the  $O_2$  supply to an increase, in cerebral  $O_2$  uptake and functional activity. While this is generally true, we have encountered exceptions in both directions and have some data by which they may be understood. Reduction in cerebral  $O_2$  supply produced by gradually progressing cerebral anemia (hemorrhage) invariably led to an increase in the A-V oxygen difference. As long as this was sufficient to compensate for the diminution in blood flow and to prevent a significant decrease in cerebral  $O_2$  uptake, little or no alteration in cerebral functional activity could be detected, but once the limit of this type of compensation had been reached and the  $O_2$  uptake began to fall, deterioration rapidly ensued (table 2). At the opposite extreme we have found two sets of circumstances under which an increase in cerebral blood flow did not lead to an increase in cerebral  $O_2$  uptake. One was the intravenous infusion of epinephrine in animals whose cerebral functions, blood flow and metabolism were optimal at the time (table 3, expts. 30 and 31); the decrease in  $O_2$  uptake actually observed is probably referable to an uneven effect on the A-V oxygen difference in different parts of the brain, that in the functionally silent areas (where the A-V difference would be decreased markedly by an increase in blood flow because the demand for  $O_2$  must be relatively small) apparently assuming dominance over that in the active regions (where the decrease in the A-V difference presumably would be just enough to compensate for the increased flow). This explanation was already put forward to account for a similar effect from  $CO_2$  inhalation in dogs whose total cerebral blood flow was maintained constant (18). It seems preferable to the alternative possibility that epinephrine depresses cerebral  $O_2$  consumption, not only because of the well-known capacity of this drug to produce central nervous excitation in man, but also because of the increase in cerebral  $O_2$  uptake produced by it when cerebral functions were depressed (table 3). Increase in cerebral blood flow also failed to increase cerebral  $O_2$  consumption in some experiments when, following hemorrhage, transfusion was delayed until respiratory failure had occurred (table 2, expt. 25). This confirms, in another animal and with other methods, the existence of a state previously designated as the "reversal", characterized by prolonged or permanent diminution in the ability of brain cells to take oxygen out of the blood and associated with a corresponding loss of cerebral functional activity (18).

Thus a decrease in cerebral blood flow need not lead to a diminution in cerebral  $O_2$  uptake if the former is small (and brief) enough to be compensated fully by increase in the A-V oxygen difference; an increase in flow does not cause an increase in  $O_2$  uptake if the existing state of cerebral function, flow and metabolism is already optimal, or if cerebral anoxia has been sufficiently severe and prolonged to interfere with the ability of brain cells to utilize oxygen (the "reversal" (18)). With these exceptions, and within these limits, cerebral  $O_2$  consumption varies directly with cerebral blood flow when the change in the latter is primary.

When the change in cerebral  $O_2$  uptake is brought about by a convulsant or narcotic drug there is also a consensual change in cerebral functional activity and blood flow, but here the alteration in cerebral blood flow probably is the result of a primary change in cerebral metabolism. Characteristic features such as dependence of the increase in  $O_2$  uptake and blood flow on increased functional activity, occurrence of a depressed phase in all three after a period of convulsions, irregular and uncertain behavior of the A-V oxygen difference, and individual variations among the convulsant drugs used, have already been presented (section 4a) and need not be repeated here. The occurrence of prolonged or permanent depression of cerebral functions and metabolism (the "reversal") following various stimulant procedures was demonstrated in earlier work (18). Our present findings indicate that the  $O_2$  supply to the brain need not be reduced at the time of the stimulation for this to occur, as then seemed to be the case, for in the present experiments anoxemia did not occur and cerebral blood flow was invariably increased during the convulsions. Apparently an increase in cerebral  $O_2$  consumption to something less than double its resting level by means of a convulsant drug (table 4) will be followed by a state characterized by depressed cerebral functional activity,  $O_2$  uptake and blood flow, which is identical with the effect of cerebral anoxia due to hemorrhage. It is interesting that nearly doubling the  $O_2$  demand had the same type of reversible depressant effect as halving the  $O_2$  supply. Since a prolonged depression of cerebral functions follows the convulsions produced by sodium cyanide, which apparently owes all these effects to reflexes from the carotid and aortic bodies (since they are lacking when the drug is given after denervation of these structures (19)), it is evident that the phenomena of post-convulsant depression do not depend entirely on a direct diphasic action by the convulsant drug on nerve cells, but are more probably related to the increased cerebral metabolic activity associated with the convulsions. Thus anoxia of the brain, with all its manifestations, can be produced quite as well by increasing the  $O_2$  requirement to exceed the available  $O_2$  supply, as by reducing the supply without altering the requirement. We have also tried convulsant drugs when ocular reflexes had disappeared and slow or absent breathing and low blood pressure gave indication of cerebral depression. With a single exception, the drugs uniformly failed to improve cerebral functional activity or  $O_2$  uptake under such circumstances. The exception was an experiment (table 4, expt. 28) in which, following a large total dose of picrotoxin, blood pressure rose, cerebral A-V oxygen difference increased, ocular reflexes returned and spontaneous breathing was resumed; no convulsions occurred, but the improvement of blood pressure, respiration and ocular reflexes, associated with an increase in the A-V oxygen difference (without significant change in cerebral blood flow) shows that a direct stimulation of nerve cells can be produced by a convulsant drug in the presence of depression. The margin between this and the onset of convulsions, leading to a more severe post-convulsant depression, may however be a narrow one. Furthermore, this instance of stimulation was altogether exceptional, probably because the factor responsible for loss of cerebral functions usually was anoxia, the effects of which would be readily removed by an increase

in cerebral blood flow (as by epinephrine—table 3) if the “reversal” had not occurred, and if it had, convulsant drugs could scarcely remedy the cellular disorganization which this implies. Our present impression is that convulsant drugs are not likely to prove beneficial in the presence of cerebral anoxia unless they lead to a rise in blood pressure. They may shorten the period of recovery following a depressant drug such as pentothal, but in our animals the stimulant effect of the analeptic was followed by a depression in cerebral  $O_2$  consumption to a level at least as low as that produced by the depressant in the first place and the total recovery period seemed to have been prolonged rather than shortened. This is in accord with the finding by Mousel and Essex (13) that analeptic drugs delay the recovery from pentothal in dogs, cats and rabbits.

The effects of pentothal on cerebral functions,  $O_2$  uptake and blood flow were the precise opposite of those of the convulsants except for the subsequent swing in the opposite direction, which did not occur with pentothal (table 4). The ability of this drug to produce a distinct diminution in cerebral  $O_2$  uptake when it is given in moderately effective dosage thus is demonstrated and the findings of Quastel and Wheatley (15) are confirmed. Recovery from the depressant effects of pentothal took place more rapidly than was the case following a comparable diminution in cerebral  $O_2$  uptake produced by hemorrhage or convulsant drugs. This suggests that the effect of pentothal is the more benign and is exerted in a different manner. If it is true that the depression following a convulsant is basically the same as that following hemorrhage and is due to cerebral anoxia created by increasing the  $O_2$  requirement more than the  $O_2$  supply, it becomes probable that a decrease in cerebral  $O_2$  uptake produced by a drug such as pentothal might increase the ability of the brain to withstand the effects of anoxia however produced. The margin of safety may be relatively narrow and the gain would be bought at the price of marked if not total interference with the capacity to perform mental and physical tasks, but for situations in which avoidance of severe cerebral damage from anoxia is a critical consideration and consciousness on the part of the subject is not essential this procedure might be beneficial. Examples would be severe poisoning by CO, marked anoxemia from respiratory obstruction, pulmonary edema, inhalation of high concentrations of  $N_2O$  or  $C_2H_4$  for surgical operations, or cerebral anemia due to low blood pressure or high cerebrospinal pressure.

*The intrinsic control of the cerebral circulation.* Figure 5 indicates not only that cerebral blood flow is automatically adjusted to the metabolic requirements of the brain, but also that the adjustment does not depend on passive effects from changes in systemic blood pressure. As to the nature of this adjustment, we can only confirm our earlier finding (6) that total cerebral blood flow in the monkey is affected more markedly and consistently by changes in  $pO_2$  than by changes in  $pCO_2$  in the arterial blood. As between the two principal metabolic gases  $O_2$  therefore appears to be the more likely mediator of an intrinsic adjustment of this type, but it is much more probable that the control is achieved by summation of all of the vasodilator products of metabolism (changes in  $pCO_2$  and  $pO_2$ , acid-base adjustments, local changes in temperature, perhaps liberation of K, histamine,

acetyl choline, etc.) and that no one agency should be singled out as being solely responsible. We have noted elsewhere (20) that the current widespread inclination toward  $\text{CO}_2$  as the main or sole factor depends on the accidental circumstance that most of the recent work in this field has been done on the circulation in the cerebral cortex of the cat. The situation there is not necessarily representative of that in the cortex of other animals, nor the situation in the cortex of any animal of that in other parts of its brain.

The vasomotor response in these experiments showed parallelism with the respiratory. In the hemorrhage experiments we were able to predict which animals would make a complete recovery by observing the behavior of their blood pressure and cerebral blood flow after the transfusion, for those in which these came back to or above the control level recovered, and those in which they did not do so failed to recover. In the single case of restoration of cerebral functions by an analeptic drug recovery of vasomotor tone began before ocular reflexes or spontaneous breathing returned. Our findings indicate that the vasomotor center is one of the most susceptible of intracranial contents to anoxic derangement, and not the most resistant, as the hypertension associated with acute elevation of intracranial pressure suggests (23). This question also is considered elsewhere (20). At present it seems quite likely that the onset of sudden collapse at high altitude is due to absence of the vasomotor response, as has already been suggested (21). If this is true for this type of cerebral anoxia it should be equally true for others, and the vasomotor center then may play the decisive rôle in determining whether cerebral functions will or will not be maintained. Loss of consciousness if the subject is in the erect position then may be viewed as a safety reaction, intended to safeguard venous return to the heart (21). If it is also associated with a diminution in cerebral  $\text{O}_2$  uptake (which should be the case in unconsciousness of any cause), this would be an added safety measure to protect the brain from  $\text{O}_2$  utilization in excess of the available  $\text{O}_2$  supply

## SUMMARY

1. Cerebral  $\text{O}_2$  metabolism has been measured *in vivo* in lightly anesthetized monkeys by measuring cerebral blood flow directly while samples of cerebral venous and arterial blood were collected for subsequent analysis. Important findings:

	MONKEY'S BRAIN ML./100 G./MIN.		$\text{QO}_2$	CORRESPONDING VALUE FOR 1400 G. HUMAN BRAIN (ML./MIN.)	
	$\text{O}_2$ uptake	Blood flow		$\text{O}_2$ uptake	Blood flow
"Normal" mean.....	3.7	47	11.1	52	660
"Normal" minimum.....	2.5	33	7.5	35	460
"Normal" maximum.....	4.5	74	13.5	63	1040
Highest of all (picrotoxin convulsion) ..	6.5	91	19.5	91	1275
Lowest with recovery (hemorrhage) ....	1.85	13.2	5.6	26	185
Lowest of all (moribund).....	0.39	5	1.17	6	70

2. Cerebral  $O_2$  uptake invariably changed in the same direction as cerebral functional activity (judged by muscular movements, ocular reflexes, character of respiration, level of blood pressure) whether the latter underwent spontaneous changes or was altered by changes in cerebral blood flow (hemorrhage transfusion, epinephrine infusion) or by convulsant (metrazol, picrotoxin, nikitamide) or depressant (pentothal) drugs. The "physiological" range of cerebral  $O_2$  uptake was from about half to nearly double the resting "normal" value. Convulsions were followed by a period of depressed  $O_2$  uptake of the same order as that produced by a deeply narcotic dose of pentothal.

3. Of the 3 possible correlations among (a) A-V oxygen difference, (b) blood flow and (c)  $O_2$  uptake of the brain, that between (a) and (b) was poorest, that between (a) and (c) somewhat better, but that between (b) and (c) by far the best. This is believed to indicate that the tone of cerebral blood vessels is automatically adjusted to the metabolic requirements of the brain. Changes in A-V oxygen difference then signify only that this adjustment has been imperfect and do not justify conclusions as to changes either in cerebral blood flow or metabolism. Previous work indicating that the cerebral circulation of the monkey is affected more consistently and strongly by changes in  $pO_2$  than in  $pCO_2$  has been confirmed; as between these,  $pO_2$  is the more likely to be responsible for the intrinsic control, though it is probable that any or all of the vasodilator products of metabolism ( $CO_2$ , anoxia, acid, heat, K, acetyl choline, histamine, etc.) may play a part.

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# THE DETERMINATION OF CEREBRAL BLOOD FLOW IN MAN BY THE USE OF NITROUS OXIDE IN LOW CONCENTRATIONS<sup>1</sup>

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Quantitative measurements of the total blood flow to the brain of man have not previously been reported and the few procedures which have been used for the purpose of obtaining relative or qualitative values have recently been subjected to considerable criticism (1).

The method to be described is based on the principle that the rate at which the cerebral venous blood content of an inert gas approaches the arterial blood content depends upon the volume of blood flowing through the brain. Certain aspects of this principle were recognized by Haggard (2) who postulated that the rate at which an anesthetic gas is taken up by the brain depends in part on the rate of blood flow to that organ, and similarly by Ferris, Molle and Ryder (3) who stated that the clearance of nitrogen from the brain during the inhalation of 100 per cent oxygen may be markedly influenced by the magnitude of cerebral blood flow. As far as we are aware, however, the principle has not previously been subjected to mathematical analysis and applied to actual measurements of cerebral blood flow in animals or man. The experiments described in the preceding report (4) afforded an opportunity for calibrating this procedure against direct measurements of cerebral blood flow and thus for refining the technical and theoretical factors to a degree that would not otherwise have been possible.

**METHODS.** The specific substance which is employed is not of consequence provided that it is physiologically inert in the concentrations employed, capable of diffusing rapidly across the blood-brain barrier, and susceptible of accurate analysis in the blood. It need not even be a gas if it meets all these requirements. In the present experiments nitrous oxide has been employed although other gases may be found to be more suitable. Radioactive gases offer certain unique advantages and their use is being considered.

In the animal experiments the inhaled gas consisted of 40 per cent nitrous oxide in oxygen which was inhaled through the inspiratory valve attached to the tracheal cannula. In the early experiments on man 15 per cent nitrous oxide and 85 per cent oxygen was employed and was inhaled through the inlet tube of an anesthesia mask. Because the presence of nitrogen in the blood introduces cer-

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tain difficulties which will be discussed later, its effects were minimized by the inhalation of 100 per cent oxygen for a period of at least 20 minutes before each determination. It is realized that the high oxygen tensions are likely to alter cerebral blood flow but these experiments were designed to aid in the development of the method rather than to establish normal values. In later experiments a mixture of 15 per cent nitrous oxide, 21 per cent oxygen and 64 per cent nitrogen was employed and the analytical error due to nitrogen was eliminated by the calculation discussed below. The 15 per cent nitrous oxide has few detectable physiological effects in most normal subjects; some individuals may experience slight dizziness or cutaneous numbness after breathing the mixture for 10 to 15 minutes. Even 40 per cent nitrous oxide has no characteristic effect on the cerebral blood flow of anesthetized monkeys as measured by the bubble flow meter.

Arterial blood was obtained by femoral puncture in man and from a femoral or carotid cannula in animals. Cerebral venous blood was collected from a metal cannula in the torcular Herophili in dogs, from both internal jugular bulbs in monkeys, and from a needle in the right internal jugular vein in man using the technique described by Myerson et al. (5). In a typical experiment in man, 19 gauge needles fitted with obturators were inserted into the femoral artery and internal jugular vein after infiltration with procain solution. The obturators were removed and each needle was connected to a manifold by means of annealed silver tubing of 1 mm. bore. Simultaneous arterial and venous samples were then obtained at intervals during 20 minutes of inhalation of the nitrous oxide-oxygen mixture. In practice it should be necessary to take only 4 such pairs of samples at 2, 4, 6 and 10 minutes. Blood samples were taken into 10 cc. syringes wetted with heparin and containing 1 cc. of mercury. These syringes were fitted with a short length of rubber tubing and a clamp and after the samples had been obtained the capillary of the tip was filled with mercury. They were then kept with the tips up in a refrigerator so that the blood was entirely sealed between mercury and glass. Analyses for oxygen and carbon dioxide were performed within 3 hours and for nitrous oxide within 24 hours. The samples were analyzed in the Van Slyke-Neill manometric apparatus by the method of Orcutt and Waters (6) with certain modifications: the blood was transferred from the syringe to the chamber of the manometric apparatus over mercury to avoid all contact with air and the reagents used were kept free of air over mercury. For the nitrous oxide analyses, carbon dioxide and oxygen were absorbed simultaneously by the addition of 2 cc. of the usual hydrosulfite-anthraquinone reagent and where 15 per cent nitrous oxide was employed 2 cc. of blood were analyzed. Analyses were done in duplicate and consistent checks within 0.05 vol. per cent were obtained with the modified technique. In these analyses nitrous oxide is not determined as such but as residual gas after absorption of oxygen and carbon dioxide. Although the error due to nitrogen can be removed by calculation, in the first 3 experiments on man and in all the animal experiments denitrogenation was employed beforehand and the nitrous oxide made up in oxygen to render the error due to nitrogen negligible.

*Derivation of a formula for cerebral blood flow.* The familiar Fick formula applied to a single organ like the brain may be expressed as:

$$CBF = \frac{100 Q_t}{(A - V)t} \quad (1)$$

where  $CBF$  represents cerebral blood flow expressed as cc./100 grams of brain/minute.

$Q_t$  represents the quantity of oxygen, expressed as cc./100 grams of brain, consumed in time  $t$ .

$A$  and  $V$  represent arterial and cerebral venous blood oxygen contents as vol. per cent.

$t$  represents any time interval in minutes.

Although the same basic formula is applicable to any substance which is removed from the blood by the brain, in the case of inhalation of nitrous oxide the arterial and venous contents of that gas both start from zero and increase with

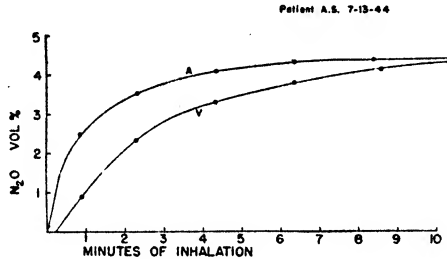


Fig. 1. Arterial and internal jugular blood concentrations of nitrous oxide in a human subject during the inhalation of 15 per cent nitrous oxide in oxygen.

time, the arterial more rapidly than the venous. The course of a typical experiment is shown in figure 1. Obviously  $A-V$  is not a constant but rises rapidly to a maximum in the first 30 seconds then decreases progressively. The amount of gas lost per 100 cc. of blood in passing through the brain cannot be calculated simply from  $(A-V)t$  as with oxygen, but is represented by the area between the curves  $A$  and  $V$  from zero time to time  $t$ , i.e.  $\int_0^t (A-V) dt$ . Equation 1 then becomes:

$$CBF = \frac{100 Q_t}{\int_0^t (A - V) dt} \quad (2)$$

Where  $Q_t$  represents the quantity of  $N_2O$  (expressed as cc./100 grams of brain) taken up by the brain from the beginning of inhalation to time  $t$ .

$A$  and  $V$  represent the  $N_2O$  content of arterial and cerebral venous blood expressed as vol. per cent.

The quantity  $\int_0^t (A-V) dt$ : This may be ascertained by direct serial determina-



tions of arterial and venous  $N_2O$  contents (as has been done in fig. 1), but if the manner in which  $(A-V)$  varies with time could be found, it could be calculated on the basis of fewer analyses. Since the rate of change of  $(A-V)$  at any specific time is determined by the rate of uptake of  $N_2O$  by the brain, which is in turn a function of the magnitude of  $(A-V)$  at that time,  $(A-V)$  should be an exponential function. Plotting  $(A-V)$  semi-logarithmically against time (figs. 2, 3, 4) it is

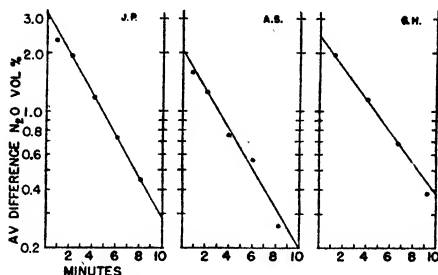


Fig. 2. Cerebral arteriovenous nitrous oxide differences in 3 patients, plotted semi-logarithmically against time of inhalation.

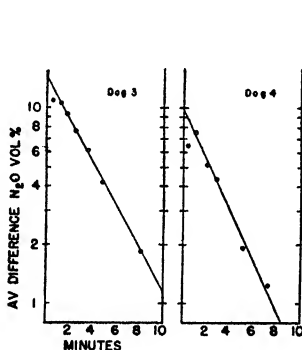


Fig. 3.

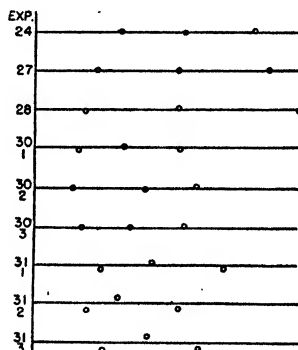


Fig. 4

Fig. 3. Cerebral arteriovenous nitrous oxide differences in 2 dogs, plotted semi-logarithmically against time of inhalation.

Fig. 4. A representation of the semi-logarithmic plot of the cerebral arteriovenous nitrous oxide difference against time in 9 determinations on monkeys. For convenience the lines have been tilted so that they lie parallel but  $P$  and  $k$  are given for each line in table 1.

seen that in each case the points fall closely upon a straight line. This confirms the expected exponential nature of the arteriovenous nitrous oxide difference which may now be expressed in the usual mathematical form:

$$(A-V) = Pe^{-kt} \quad (3)$$

$P$  represents the theoretical value of  $(A-V)$  at zero time and may be obtained from the semi-logarithmic graph by extrapolation to zero time.  $k$  which repre-

sents the slope of the straight line semi-logarithmically plotted is obtained from intercepts of the line as follows:

$$k = \frac{\log (A - V)_{t_1} - \log (A - V)_{t_2}}{(t_2 - t_1) \log e} \quad (4)$$

Integrating equation 3 from times 0 to  $t$  results in the following expression:

$$\int_0^t (A - V) dt = \frac{P}{k} (1 - e^{-kt}) \quad (5)$$

Since the arterial and venous content of  $N_2O$  are zero at the beginning of the inhalation,  $(A - V)$  for  $N_2O$  does not start immediately at a maximal value but rather rises sharply from zero to a maximum in the first half minute and then decreases exponentially. This divergence from a true exponential form is small and readily corrected for. As seen in figure 5, the actual integral is less than the integral of the simple exponential *OPRS* by the small triangle *NOP* the area of

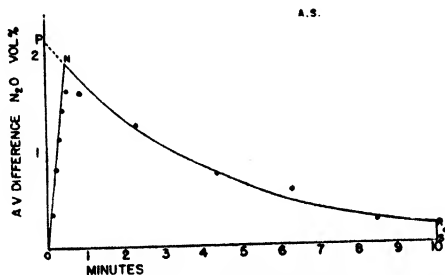


Fig. 5. Cerebral arteriovenous nitrous oxide differences in the subject of figure 1 plotted against time. Open circles represent values obtained by analysis, closed circles represent values obtained in the first minute from the extrapolated arterial and venous curves. The closeness with which the calculated integral (*ONRS*) fits the experimental values is shown.

which is equal to  $P/4$ . Thus by subtracting this from the integral derived in equation 5 the true integral is closely approximated (without this correction the error is only about 6 per cent, when corrected the error becomes even smaller). Thus the corrected integral is obtained:

$$\int_0^t (A - V) dt = \frac{P}{k} (1 - e^{-kt}) - \frac{P}{4} \quad (6)$$

The closeness with which this calculated integral fits the data can be seen in figure 5. In the three human experiments (G. H., A. S., and J. P.)<sup>2</sup> this calcu-

<sup>2</sup> In these three experiments 8 pairs of samples were taken in order that a valid comparison could be made between the graphic and calculated integrals. The remaining 13 determinations on human beings were based upon the simplified procedure using only 4 pairs of samples. In many experiments there is a tendency for the  $A - V$  curve to deviate slightly from that of a simple exponential in the region  $t = 10$  minutes. This is evidence that there is more than one nitrous oxide absorbing phase in the brain. This deviation, however, is slight and it occurs in a region where its effect on the entire integral is negligible, so that there is consistently good agreement between values for the integral obtained graphically and those calculated from the exponential formula (col. 4 and 5, Table 2).

lated integral is 104 per cent, 101 per cent and 104 per cent of the graphic integral respectively.

*The quantity  $Q_t$ :* The amount of nitrous oxide absorbed by the brain from the beginning of inhalation to time  $t$  is calculable by assuming that the mean brain tension of the gas is equal to its tension in the blood leaving the brain. If this assumption were correct then:

$$Q_t = V_t S \quad (7)$$

Where  $V_t$  = nitrous oxide content of cerebral venous blood as vol. per cent at time  $t$ .

$S$  = ratio of solubilities of  $N_2O$  in brain and blood, i.e.,  $\frac{\alpha \text{ brain}}{\alpha \text{ blood}}$  where solubility is expressed as cc.  $N_2O$ /cc. blood and cc.  $N_2O$ /gram brain at 760 mm.  $N_2O$  tension and  $38^\circ$ .

Although the assumption that the mean brain tension of nitrous oxide is equal to the cerebral venous tension is only approximately true in the first few minutes of inhalation, it becomes increasingly valid as equilibrium progresses to completeness. It is now pertinent to determine the time after which the assumption is sufficiently valid that its use introduces no appreciable error into the calculation.

By substituting in equation 2 the values for  $Q_t$  and  $\int_0^t (A-V)dt$  obtained in equations 6 and 7 respectively, and rearranging, the following relation appears:

$$\frac{CBF}{S} = \frac{100 V_t}{\frac{P}{k} (1 - e^{-kt}) - \frac{P}{4}} \quad (8)$$

Throughout the course of 10 or 20 minutes of nitrous oxide inhalation the left hand member of equation 8 should remain constant whence the constancy of the right hand member constitutes a test for the validity of the assumption expressed in equation 7. These calculations have been made in the case of the three detailed human experiments and in the two experiments on dogs and the results are

presented in figure 6. It is seen that in each case the quantity  $\frac{100 V_t}{\frac{P}{k} (1 - e^{-kt}) - \frac{P}{4}}$

decreases somewhat throughout the early periods of inhalation but appears to reach a fairly constant value in about 10 minutes, after which it decreases only a few per cent over protracted periods. From this it is inferred that mean brain tension of nitrous oxide at first is somewhat lower than the corresponding cerebral venous tension but that after 10 minutes they have come sufficiently close to make the assumption of equation 7 sufficiently valid for present purposes.<sup>3</sup>

<sup>3</sup> It is now possible to calculate for each of the earlier  $V_t$ 's a factor ( $r$ ) by which it must be multiplied in order that the quantity  $\frac{100 V_t r}{\frac{P}{k} (1 - e^{-kt}) - \frac{P}{4}}$  may at all times be equal to the

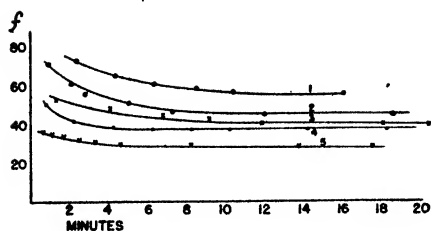


Fig. 6. The function  $\left[ \frac{100 V_t}{\frac{P}{k}(1 - e^{-kt}) - \frac{P}{4}} \right]$  plotted against time for experiments on 2 dogs and 3 human subjects. After 10 minutes it is practically constant in each case. Curve 1—patient A. S., 2—dog 4, 3—patient G. H., 4—patient J. P., 5—dog 3.

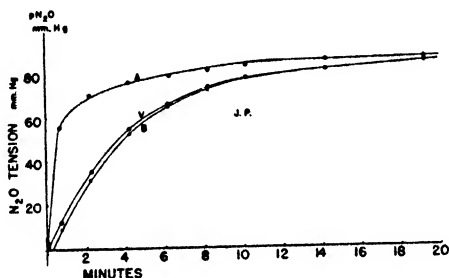


Fig. 7. Curves showing the arterial and internal jugular blood tensions and the mean brain tensions of nitrous oxide in a human subject during inhalation of 15 per cent nitrous oxide in oxygen. Values for mean brain tensions were calculated as explained in the text.

Equation 2 with proper substitutions from equations 6 and 7 now becomes the working formula for calculating cerebral blood flow by this method:

$$CBF = \frac{100 V_t S}{\frac{P}{k}(1 - e^{-kt}) - \frac{P}{4}} \quad (9)$$

where  $t$  is 10 minutes or greater.

final constant value of  $\frac{100 V_t}{\frac{P}{k}(1 - e^{-kt}) - \frac{P}{4}}$ . This factor ( $r$ ) is then the ratio of brain nitrous

oxide tension to venous nitrous oxide tension and from it the mean brain tension at each time may be calculated. In figure 7 may be seen the curves for arterial, internal jugular and mean brain tensions of  $N_2O$  for one of the human subjects calculated in this manner. The rate at which mean brain tension of  $N_2O$  approaches that in internal jugular blood is a function of cerebral blood flow and, for comparable blood flows, an index of the "vascularity" of that particular brain, the latter term representing the proportion of vascular diffusion surface to weight of brain tissue. Such an index may have some clinical significance.

The necessary data are obtained in human subjects as follows: simultaneous arterial and internal jugular blood samples are obtained at approximately 2, 4, 6 and 10 minutes following the onset of inhalation of the nitrous oxide gas mixture. The times of taking the samples are accurately measured and the respective arteriovenous nitrous oxide differences are plotted semilogarithmically against time. A best fitting straight line through the earliest three points (where the arteriovenous differences are sufficiently large that analytical error is inappreciable) determines  $P$  and  $k$ . The  $t$  in equation 9 is the time of the last venous sample and should be 10 minutes or longer.

The solubility factor  $S$  remains to be considered. It has not yet been possible to determine this factor to a great degree of accuracy. Early attempts to determine it by equilibration of brain tissue homogenates *in vitro* yielded the unexpected finding that the apparent solubility of nitrous oxide in brain decreased significantly with the time of equilibration at 38°. It was concluded that changes take place in brain tissue so treated which alter its capacity for absorbing nitrous oxide and that reliable determinations could be carried out for the present only *in vivo*. For this two different techniques have been employed. In one, a representative sample of brain tissue was removed anaerobically from an anesthetized dog after a protracted period of nitrous oxide inhalation and its content of this gas determined and compared with that of torcular blood obtained simultaneously. The obvious technical difficulties of this procedure have not yet been fully mastered; the values for  $S$  thus obtained in 4 different animals were 1.0, 1.0, 1.3 and 1.4, respectively.

Another method is presented in the direct measurement of cerebral blood flow in the monkeys used in the experiments just reported (4) simultaneously with the administration of nitrous oxide and the collection of the necessary blood samples. From the data thus secured  $S$  may be calculated by a rearrangement of equation 8:

$$S = \frac{CBF \left[ \frac{P}{k} (1 - e^{-kt}) - \frac{P}{4} \right]}{100 V_t} \quad (10)$$

The values for  $S$  thus obtained are presented in table 1. The fairly wide range of values found is probably not evidence that the solubility of nitrous oxide in different brains of the same species varies widely (there is as wide a variation in different determinations on the same animal as between different animals) but is indicative of certain technical difficulties which have not yet been eliminated. The average value for  $S$  thus obtained (1.3) compares well with that obtained by the other quite different method in dogs and has been used as a first approximation in the calculations of cerebral blood flow here presented. Since it is hardly likely that the solubility coefficient of nitrous oxide in human brain would vary significantly in the same individual in the course of an hour or so, the method in its present form is applicable to the quantitative measurement of changes in cerebral blood flow induced by various procedures, and if the further assumption that this coefficient will not vary appreciably from one brain to another is tenable

the method may be used to study deviations in the blood flow in different pathological states. Although the true value for  $S$  is not expected to lie far from the tentative value of 1.3, the precise evaluation of the absolute cerebral blood flow in man must await more accurate determination of this factor and a knowledge of its variability.

A critical examination of the method here proposed brings to light several considerations which must be evaluated before the procedure can be regarded as yielding absolute and reliable figures for total cerebral blood flow in man. A discussion of some of these seems pertinent:

1. The derivation assumes that the brain is a homogeneous system with respect to blood flow and nitrous oxide capacity, yet the presence of at least two discrete tissue masses in the brain (white and grey matter) and the cerebrospinal fluid raises a question as to whether such an assumption is warranted. To ascertain the effects of differences in blood flow and in nitrous oxide capacity in different regions of the brain, theoretical biphasic and triphasic schemata have been set up representing variations in rates of blood flow, weights of tissue and nitrous oxide capacity of greater magnitude than are likely to occur in reality. Calculations on these schemata reveal the following: *a.* Where the variations among the phases are considerable the combined ( $A-V$ ) is no longer a simple exponential function and its rate of decrease ( $k$ ) instead of remaining constant diminishes with time. The fact that in the experiments which have thus far been performed in animals and man the values for ( $A-V$ ) conform reasonably well to simple exponential functions is an indication of the relative homogeneity of these brains. *b.* Notwithstanding their more complex nature if these ( $A-V$ ) functions of heterogeneous systems are treated as simple exponentials by the use of a best fitting straight line through their semi-logarithmic plot at 2, 4 and 6 minutes, values for  $P$  and  $k$  thus obtained yield a calculated flow within 15 per cent of the true flow. *c.* Even for the most heterogeneous systems the mean flow is accurately calculable by using the true integral for  $A-V$  instead of the integral based on the simple exponential. \*Experimentally such a true integral could be closely approximated by more frequent blood samples or by the continuous withdrawal of blood from the artery and vein at a slow constant rate over a period of ten minutes. Unless subsequent studies yield results markedly different from these early experiments, such procedures seem unnecessary and the brain can for practical purposes be considered homogeneous.

2. In order to obtain true cerebral blood flow by this method it must be assumed that the blood samples from the right internal jugular vein represent mixed cerebral venous blood. The anatomical fact that the torcular in man is often incomplete does not necessarily imply that blood from one jugular is not a representative sample. Riggs (7) has found in an examination of 25 autopsy specimens that torcular blood is distributed both to the right and left lateral sinuses in 15, usually with a preponderance to the right, in 9 cases the torcular communicated entirely with the right and in one case entirely with the left lateral sinus. Gibbs and Gibbs (8) have found in a study of flows in 24 autopsy specimens that on the average 95 per cent of right lateral sinus blood is derived from

TABLE 2

*Cerebral blood flow and cerebral oxygen consumption in eleven patients determined by the proposed method*

SUBJECT	DIAGNOSIS	t min.	$\int_0^t (A-V)_{N_2O} dt$		$V_t$ N <sub>2</sub> O vol. %	A-V O <sub>2</sub> vol. %	CEREBRAL BLOOD FLOW cc./100 g./min.	CEREBRAL O <sub>2</sub> CON- SUMPTION cc./100 g./min.
			by formula	graphi- cally				
*G. H. BF 44	Multiple sclerosis	11.92	11.1	10.7	4.43	6.2	52	3.2
*A. S. BF 26	Essential hypertension	10.30	7.5	7.4	4.26	4.0	74	3.0
*J. P. BF 29	Gastric neurosis	10.22	11.4	11.0	4.27	10.2	50	5.1
W. G. WM 23	Normal	10.50	9.3	8.4	4.72	6.6	66	4.4
J. F. BM 46	Essential hypertension	10.25	7.9	8.1	4.49	4.6	74	3.4
V. L. WM 42	Hypochromic anemia	9.97	10.0	9.7	4.30	5.3	56	3.0
	Repeat 15' later	10.03	8.7	8.9	3.81†	5.5	57	3.1
L. F. WF 30	Chronic P.I.D.	8.00	6.0	5.5	2.73	6.2	60	3.7
	Repeat 15' later	8.00	10.5	9.8	4.13†	6.9	52	3.6
P. G. WM 55	Gastric neurosis	9.90	†	8.70	2.71	7.5	41	3.0
	Repeat 15' later	9.94	†	3.86	1.24†	7.6	42	3.2
J. T. WM 35	Convalescent monarthrititis	9.87	7.5	7.5	4.31	5.3	75	3.9
R. O. BM 20	Convalescent pneumonia	10.15	†	5.5	3.01	4.7	71	3.3
	Repeat 15' later	10.05	†	3.6	2.16†	5.3	78	4.1
L. H. WF 20	Convalescent rheumatic fever	9.92	8.3	8.0	4.29	7.0	67	4.7
	Repeat 15' later	10.03	6.9	6.7	4.10†	5.7	77	4.4
Mean ¶ .....						6.2	62	3.7
"Normal" values for monkey obtained by means of the bubble flow meter, 11 observations (4), mean .....						8.0	47	3.7

\* In the first 3 determinations the gas mixture employed was 15 per cent nitrous oxide and 85 per cent oxygen and was preceded by 30 minutes' inhalation of 100 per cent oxygen. The remaining determinations were made with a mixture containing 15 per cent nitrous oxide, 21 per cent oxygen and 64 per cent nitrogen and the subject breathed room air before the determination.

† In these two patients a poorly fitting mask produced an eccentric curve of arterial nitrous oxide content with the result that the  $\Delta V$  difference was not exponential and the formula could not be used. It was still possible to obtain the integral graphically.

‡ Where determinations were repeated on the same individual 15 minutes later there was still a small amount of nitrous oxide present in the brain and venous blood as determined in blood samples taken immediately before the second period of inhalation. For these determinations  $V_t$  represents the difference between the final and the small initial venous concentration.

¶ The authors do not attach any special significance to the mean values in man representing as they do a heterogeneous group. They are included merely as an indication of values to be expected in man with no gross derangements in cerebral function.

The identity of the mean values for cerebral oxygen consumption in man and monkey must be construed as fortuitous, since apprehensiveness and disease in the human subjects and light anesthesia and the operative procedures in the monkeys would be expected to modify the results. That the values obtained by these two different methods are of the same order of magnitude is, however, certainly significant.

determination, 9 measurements have been performed on rhesus and spider monkeys for comparison with simultaneous cerebral blood flow determinations obtained directly by means of the bubble flow meter (12). A value of 1.3 obtained from these data as described previously has been assigned to  $S$ . The comparison is presented in table 1. Over a wide range of blood flows (17 to 76 cc./100 g./min.) there is good agreement between the direct measurements and those calculated by the present method. The mean deviation of the calculated values from the direct measurements is  $\pm 10$  per cent. Technical difficulties peculiar to this preparation would tend to make the error greater than that which would occur in man where blood loss resulting from the sampling would be negligible and where venous samples could be taken rapidly without fear of drawing blood from regions other than the brain. It is fair to state that not many indirect clinical measurements in widespread use today have been subjected to the rigorous test of comparison with direct measurement.

By means of equation 9 and using the value for  $S$  of 1.3 the cerebral blood flow and cerebral oxygen consumption have been calculated for 16 determinations in 11 human subjects.<sup>4</sup> The data are presented in table 2. It is worthy of note that these values for cerebral blood flow and oxygen consumption per 100 grams of brain are in excellent agreement with those obtained in this laboratory in the rhesus monkey by an entirely different method (4). These 16 experiments on human subjects are included here to indicate the feasibility of applying the method here described to clinical investigation.

#### SUMMARY

A method is described applicable to unanesthetized man for the quantitative determination of cerebral blood flow by means of arterial and internal jugular blood concentrations of an inert gas during the first ten minutes of its inhalation in low concentration.

Certain necessary assumptions are experimentally tested and results of the method in monkeys are compared with those obtained simultaneously by direct measurement of cerebral blood flow.

Sixteen determinations of cerebral blood flow on eleven human subjects by this method have thus far been made and suggest the feasibility of applying this method to clinical investigation.

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# INFLUENCE OF THE CONTACTING SURFACE ON THE COAGULABILITY AND ANTICEPHALIN ACTIVITY OF NORMAL AND HEMOPHILIC PLASMAS

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It has been known for some time that the coagulation of blood is accelerated by contact with certain types of surfaces. The hypercoagulability developed in blood after contact with a surface like glass, has been attributed to an effect on the blood corpuscles themselves (the platelets principally) as well as on the plasma colloids (1). "Cell free" plasma itself is rendered hypercoagulable by contact with glass (2) (3), though the possibility remains that in whole blood the stability of the platelets is also affected. Just how the mechanism of coagulation is altered by contact has remained obscure. It is generally agreed that, whatever takes place, influences chiefly that stage of coagulation preceding the formation of thrombin; the rate of transformation of fibrinogen into fibrin by thrombin does not seem to be modified by the type of surface contacting the plasma (1).

Normal plasma, collected with especial precautions, has the property of reducing the clot accelerating action of homologous brain extracts (4). This activity of human plasma seems to be exerted on the cephalin fraction of the thromboplastic lipoprotein (5) and may be properly designated as anticephalin activity. It appears from the following observations that plasma placed in contact with certain surfaces (e.g., asbestos, clay, glass) loses this activity rapidly as its coagulability increases. The well known difference between the rate of coagulation of blood in vessels with walls of varied composition (e.g., glass and paraffin) appears likewise to be related to the anticephalin content of the plasma. When there is an excess (as in hemophilic plasma), the difference is most pronounced; in normal plasma the difference becomes less, and it may cease to be evident when anticephalin is reduced or removed by contact with adsorbents.

**METHODS.** Citrated "cell free" plasma was separated from blood collected with especial precautions (4) from normal and hemophilic men. Pipettes used for measuring plasma were coated on the inside with a thin film of collodion. The error of measurement introduced by such a single, uniformly made thin coating is less than 0.5 per cent (mercury calibration). Clotting tubes (13 mm. i.d. x 60 mm. length) made of glass (pyrex), "Lusteroid"<sup>1</sup> or glass coated with paraffin, collodion or "Acryloid"<sup>2</sup> were used. Since the readings of determinations on paraffin, acryloid, collodion and lusteroid tubes were substantially alike, only the results on glass and lusteroid tubes are included in the tables.

Cephalin was prepared by extracting acetone dried human brain with ethyl

<sup>1</sup> Plastic tubes made by the Lusteroid Company, South Orange, N. J.

<sup>2</sup> Liquid resinous plastic produced by Rohm & Haas, Philadelphia.

ether (5). The clear extract was evaporated to a small volume, cold absolute ethanol was added and the precipitate thrown down by a short period of centrifugation. The cloudy supernatant fluid was discarded, the white sediment was suspended in a little ether and reprecipitated with cold ethanol. The precipitation was repeated once more before washing the lipid in cold acetone. The lipid was suspended in 10 cc. of 0.85 per cent NaCl as soon as all traces of the acetone had been removed and before the lipid turns yellow and adheres to the wall of the container. The temperature of the vessels and solutions was kept at about 5°C. during these steps. The best results are conveniently obtained with preparations 0.1 cc. of which will clot 0.3 cc. of recalcified normal plasma in 80 to 100 seconds and 1 cc. of normal blood in 100 to 120 seconds in 13 mm. wide glass tubes at 38°C. When kept at 5°C., cephalin suspensions have maintained their potency for 6 to 8 weeks. Slower acting cephalins usually result from extraction of powdered brains older than two months. The lipid suspension was delivered into the clotting tubes by means of an automatic pipette (6) which may be regulated to eject a uniform amount directly into the clotting mixture without loss on the sides of the tube. "Platelet extract" results from the extraction for 24 hours at 5°C. with distilled H<sub>2</sub>O (4 cc.) of approximately 0.5 cc. of packed human platelets separated by fractional centrifugation (7). The extract was centrifuged and its NaCl concentration adjusted before using. Detection of the end point in coagulation of dilute plasma specimens is rendered easier by using fluorescent light and a dark screen for a background. Plasma anticephalin activity was estimated by noting the effect on the clot accelerating property of a cephalin suspension, of incubation of the plasma with the lipid in stoppered tubes, for a fixed period before recalcification. Close attention must be paid to the order of addition of the reagents (5). Prothrombin was measured by the one-stage (8) and two-stage methods (9) using a saline extract of human brain and (in the two-stage method) waiting three minutes for full conversion of the prothrombin. In order to be able to express prothrombin time in terms of per cent of normal, titration curves were worked out for both methods at various dilution levels of pooled normal plasma. Unless otherwise specified, statements regarding prothrombin concentration refer to determinations by the two-stage method.

1. *The coagulation of plasma on surfaces of different composition.* Normal or hemophilic plasmas may take three or more times as long to coagulate in lusteroid as in glass tubes (table 1). The clot decelerating effect of incubation of plasma with cephalin is better demonstrated in lusteroid tubes. While the difference between the clotting time of unincubated normal plasma and that incubated 20 minutes is only 53 seconds in the glass vessel, it is 156 seconds in the lusteroid vessel. The differences are greater when hemophilic plasma is used. After plasma has been allowed to remain in contact with material like asbestos, it shows relatively little change after incubation, and clots at about the same time whether in glass or lusteroid tubes, with or without the addition of cephalin (table 1). Increasing the glass surface exposed to contact accentuates the change in the plasma. For example, normal plasma held in long glass pipettes for one hour at 20°C. may clot in about one half the time of plasma kept in paraffin coated vessels.

The hypocoagulability induced by incubation is partly due to changes in the plasma itself. Standing in a stoppered tube at 38°C. leads, after thirty minutes, to a prolongation in clotting time, a diminution in prothrombin and a rise in the pH of undiluted plasma, especially marked after thirty minutes (table 2). The change in clotting time is better detected in lusteroid tubes since the effect is not

TABLE 1

*The clotting time in glass and lusteroid tubes of normal, hemophilic and adsorbed plasmas and the effect of incubating (38°C.) the plasmas with cephalin before recalcification*

	NORMAL		HEMOPHILIC		ADSORBED*	
	Glass	Lust.	Glass	Lust.	Glass	Lust.
Cephalin clot. time† (secs.)						
0' incub.....	73	198	238	618	26	30
5' incub.....	96	198	274	1154	28	34
10' incub.....	115	235	310	1768	33	39
20' incub.....	126	354	345	2296	40	42
40' incub.....	134	506	512	>7200	43	46
60' incub.....	168	703	920	>7200	50	64
Plasma clot. time‡ (secs.).....	335	1020	2520	>7200	270	278

\* Plasma (hemophilic) in contact with asbestos fibers (10 mgm. asbestos/ml. plasma) for 2 hours at 20°C. Prothrombin content (2-stage meth.) 70% normal.

† 0.3 cc. plasma, 0.1 cc. cephalin (incubation), 0.1 cc. 0.074 M CaCl<sub>2</sub>.

‡ 0.3 cc. plasma, 0.1 cc. 0.074 M CaCl<sub>2</sub>.

TABLE 2

*Effect of standing in stoppered lusteroid tubes at 38°C. on the cephalin clotting time, prothrombin and pH of undiluted and diluted normal plasma*

PERIOD OF STANDING	100% PLASMA					20% PLASMA		10% PLASMA	
	Cephalin clot time*		Prothrombin time		pH	Cephalin clot. time*			
	glass	lust.	1-stage meth.	2-stage meth.		glass	lust.	glass	lust.
	min.	sec.	sec.	sec.		sec.	sec.	sec.	sec.
0	73	208	12	14	7.6	83	134	136	162
10	61	231	12	14	7.8	74	129	102	148
20	60	246	12	14	7.95	63	138	85	149
40	78	337	13	15	8.1	60	143	84	166
60	96	461	14	16	8.23	67	149	89	170
90			15	18	8.42				

\* 0.3 cc. plasma, 0.1 cc. cephalin, 0.1 cc. CaCl<sub>2</sub>.

offset by the clot accelerating action of contact with glass. Up to 20 minutes, however, little impairment is detected in the coagulability of undiluted plasma. Diluted (20 per cent) plasma shows little or no change up to 60 minutes. It seems wise, therefore, when estimating anticephalin activity, to restrict the incubation to a period no longer than 20 minutes and to employ lusteroid tubes, or vessels with similar properties.

2. *The coagulability of plasma after contact with various materials.* Because of the ease with which plasma can be removed from asbestos wool fibers, this adsorbent was used in most of these experiments. Similar results were obtained with kaolin and infusorial earth which, however, must be removed by centrifugation after the period of exposure is over. In tables 3 and 4 are detailed the changes that take place in normal and hemophilic plasmas exposed to contact with an adsorbent. A significant reduction in the cephalin clotting time of hemophilic plasma may take place after only 5 minutes of contact. Hemophilic plasma reaches its highest degree of coagulability after three hours' contact, whereas normal plasma requires two hours or less. The hypocoagulability that

TABLE 3

*Effect of exposure of normal plasma to contact with asbestos fibers (1 ml. plasma/10 mgm. asbestos in stoppered paraffin coated tubes at 20°C.)*

	DURATION OF CONTACT										
	Minutes			Hours							
	0	15	30	1	1½	2	3	4	8	12	24
Cephalin clot. time* (secs.).....	81	67	59	42	35	26	37	54	208	234	456
Cephalin clot. time: Mixed plasmas† (secs.).....		79	72	67	66	60	55	74	81	105	101
Plasma clot. time‡ (sec.)	607	505	423	343	410	380	351	1150	>7200	>7200	>7200
One-stage prothr. time: Seconds.....	12	12	10	9	9	10	12	31	42	71	138
% of normal .....	100	100	>100	>100	>100	>100	100	13	10	5	3
Two-stage prothr. time: Seconds.....	16	16		22		28	68		384	>900	
% of normal .....	100	100		73		60	25		4	<1	
Fibrinogen: Mgm./100 cc. plasma.	289	286	290	174	172		104	113	93	91	72
% of original amt. ....		98	100	60	59		36	39	32	31	25

\* 0.3 cc. plasma, 0.1 cc. cephalin, 0.1 cc. 0.074 M CaCl<sub>2</sub>.

† 0.25 cc. intact plasma, 0.05 cc. of adsorbed (15, 30, etc. mins.) plasma, 0.1 cc. cephalin, 0.1 cc. 0.074 M CaCl<sub>2</sub>.

‡ 0.3 cc. plasma, 0.1 cc. 0.074 M CaCl<sub>2</sub>. Glass tubes employed throughout these tests.

follows after four hours results from a decrease in prothrombin and eventually of fibrinogen itself. The decrease in prothrombin seems to progress more rapidly in normal than in hemophilic plasma. The discrepancy between results of determinations by the one-stage and two-stage methods in the first few hours of contact appears to be due to increases in the conversion rate of prothrombin, as anticephalin is progressively removed from the plasma (10). After five minutes of contact with asbestos, hemophilic plasma can accelerate slightly the clotting of intact plasma. Both normal and hemophilic plasmas are most active, in this respect, after about two hours of contact and seem to lose this property gradually as prothrombin decreases below 50 per cent (tables 3, 4). The clot accelerating

properties of adsorbed plasma might result from its content of highly convertible (anticephalin-free) prothrombin, or a substance closely associated with it.

TABLE 4

*Effect of exposure of hemophilic plasma to contact with asbestos fibers (1 ml. plasma/10 mgm. asbestos in stoppered paraffin coated tubes at 20°C.)*

	DURATION OF CONTACT												
	Minutes				Hours								
	0	5	15	30	1	2	3	4	8	12	16	24	48
Cephalin clot. time* (sec.)	212	148	84	63	46	39	28	35	65	128	167	255	660
Ceph. clot. time—mixed plasmas† (sec.)		197	138	131	94	98	97	103	107	176		227	236
Plasma clot. time:‡ (mins.)	43	31	25	21	18	16.2	5.8	5.6	13	>120	>120	>120	>120
One-stage prothr. time seconds	12	12	11	11	10	10	8	9	13	32	46	56	160
% of normal	100	100	>100	>100	>100	>100	>100	>100	85	14	12	6	3
Two-stage prothr. time seconds	16		19			22	27	30	60	270		>900	
% of normal	100		84			73	62	56	28	6		<1	
Fibrinogen:													
mgm./100 cc. plasma	490	496	506	447	338		289		258			175	88
% of original amt.				91	69		58		52			35	17

\* 0.3 cc. plasma, 0.1 cc. cephalin, 0.1 cc. 0.074 M CaCl<sub>2</sub>.

† 0.25 cc. intact plasma, 0.05 cc. of adsorbed (5, 15, or 30, etc. mins.) plasma, 0.1 cc. cephalin, 0.1 cc. 0.074 M CaCl<sub>2</sub>.

‡ 0.3 cc. plasma, 0.1 cc. 0.074 M CaCl<sub>2</sub>.

Glass tubes employed throughout these tests.

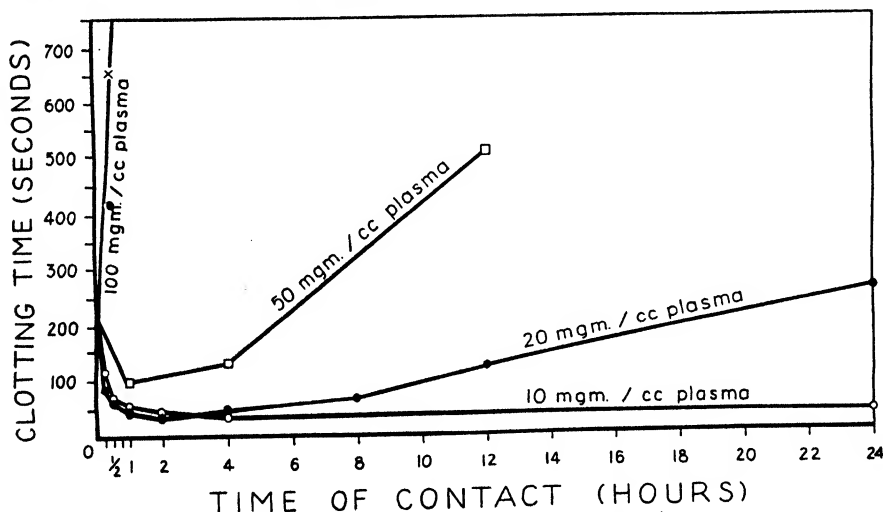


Fig. 1. The cephalin clotting time (glass tube) of hemophilic plasma after contact with increasing amounts of asbestos wool fibers.

Increasing the amount of adsorbing surface brings about these changes more rapidly. If enough adsorbent (100 mgm. per 1 ml. plasma) is used there may be a reduction in the prothrombin to 5 per cent or less, after only one minute of contact. The cephalin clotting time is then prolonged from the start (fig. 1).

The gradual decrease in prothrombin and fibrinogen when small amounts of adsorbent are used is probably due at first to adsorption. After contact for about two hours, some clotting occurs spontaneously in the plasma; part of the decrease in the prothrombin and fibrinogen that takes place then, must result from their transformation into thrombin and fibrin. As observed microscopically, plasma in contact with asbestos fibers may stand for about one hour without any evidence of fibrin formation. The fibrin first appears immediately in contact with the asbestos strands, indicating perhaps that it is in this zone first that the plasma becomes unstable.

In the order of their effectiveness, the following materials (10 mgm. per ml. plasma for two hours at 20°C.) are active in enhancing the coagulability of plasma brought into contact with them: asbestos fibers, infusorial earth, kaolin, pumice stone, filter cell, talc, glass particles and glass wool. Animal charcoal, permutit and silica gel, seemed less effective and cellophane strips, cotton fibers, iron, aluminum, copper and brass shavings have no significant effect. Partial immersion of a Pasteur-Chamberland filter candle in "cell free," citrated normal plasma for one hour reduces to one third or less the clotting time and anticephalin activity of the plasma. Filtering 10 cc. of plasma through a Berkefeld V filter over a period of forty seconds removes some of its prothrombin and so alters the plasma that on addition of cephalin it clots faster than the unfiltered plasma with normal prothrombin content. The amount of calcium required for optimal recalcification is not changed after contact of plasma with asbestos, indicating that there has been no loss in citrate. Plasmas exposed to adsorbents are, nevertheless, quite unstable. After standing for 1 to 2 hours, even at 5°C., plasmas filtered through Berkefeld candles or Seitz pads begin to clot spontaneously. It was this complication that forced British workers (11) to resort to especial methods (blocking the conversion of prothrombin by raising the pH of the medium) to insure the fluidity of filtered plasma. The often observed spontaneous conversion to thrombin of purified prothrombin solutions (12), even in the absence of calcium, may result from loss of the protection afforded by anticephalin against the effects of contact.

3. *The coagulability of diluted plasma in vessels of different composition.* When normal and hemophilic plasmas are diluted, their coagulability is enhanced, until a concentration of about 20 per cent of the plasma is reached; beyond that point there is usually a prolongation (table 5). Up to 20 per cent concentration the effect of prothrombin diminution is apparently offset by the reduction of anticephalin and the consequent increase in the prothrombin conversion rate (10). The clot accelerating effect of dilution is best observed in lusteroid tubes and is especially pronounced in hemophilic plasma. Dilution must also reduce the antithrombin content of the plasma, thereby contributing to further shortening of the clotting time. Plasma tested after being exposed to adsorbents (10 mgm./1 ml. plasma for 2 hrs. at 20°C.) does not, however, seem to have lost antithrombin. Unlike normal and hemophilic plasma, dilution of the adsorbed plasma prolongs its clotting time from the outset, and does not alter its behavior in the two types of vessel. Adsorbed plasma clots at approximately the same

rate in glass or lusteroid tubes and the effect of incubation with cephalin (20 min.) is not striking.

A certain correspondence appears to exist between the differences in the cephalin clotting time in glass and lusteroid tubes and those between unincubated and incubated plasma in lusteroid tubes (table 6). Both differences are greatest in hemophilic plasma. As anticephalin activity diminishes, the difference between the clotting time in the two tubes also progressively decreases, until it is no longer evident at the end of two hours of contact (table 6).<sup>1</sup> Since reduction of anticephalin activity by diluting plasma or exposing it to adsorbents is manifested by decreases in the difference between the clotting time in glass and lusteroid vessels, the extent of this difference may give an approximate measure of this activity.<sup>2</sup>

TABLE 5

*Effect of dilution on the clotting time† in glass and "lusteroid" tubes of normal, hemophilic and adsorbed plasmas before and after incubation with cephalin*

CONC. OF PLASMA*	TYPE OF VESSEL	NORMAL					HEMOPHILIC					ADSORBED‡				
		Period of incubation (mins.)														
		0	10	20	40	60	0	10	20	40	60	0	10	20	40	60
%		Clotting time in seconds														
100	Glass	73	115	126	134	168	238	310	345	512	920	26	33	40	43	50
	Lust.	198	235	354	506	703	618	1768	2296	>7200	>7200	30	39	42	46	64
50	Glass	65	82	97	112	146	174	216	259	318	360	34	45	57	60	64
	Lust.	154	194	223	429	617	397	543	890	1387	2400	34	46	55	66	75
20	Glass	56	67	78	95	122	254	280	330	354	390	48	55	67	71	78
	Lust.	134	173	185	294	406	331	436	457	607	758	45	52	69	78	83
10	Glass	91	100	126	134	151	293	329	340	363	378	80	91	100	106	115
	Lust.	162	187	209	255	310	402	426	470	540	657	79	89	103	108	115
5	Glass	154	169	203	210	215	400	418	450	461	473	155	162	173	180	187
	Lust.	262	274	327	337	360	487	539	565	581	622	149	156	168	179	186

\* Dilutions of plasma made with 0.85% NaCl.

† 0.3 cc. plasma, 0.1 cc. cephalin (incubation), 0.1 cc. CaCl<sub>2</sub>.

‡ Hemophilic plasma exposed to asbestos fibers for 2 hours at 20°C. (10 mgm. asbestos/ml. plasma). Prothrombin content (2-stage method) 70% of normal.

The effect of surface contact and dilution on the behavior of the plasma is best demonstrated when platelet extracts are used as the thromboplastic agent (table 6 and fig. 2). Though such extracts seldom have the potency of either cephalin or saline tissue extracts, they seem to be especially susceptible to the clot decelerating action of hemophilic plasma. It has not been possible, however, to demonstrate in glass or lusteroid tubes any clot delaying effect of incubation of platelet extracts with normal plasma. The presence of some intact platelets in the extract may nullify (in normal plasma) any clot delaying effect of the incubation. Dilution of plasma or its exposure to adsorbents enhance the clot accelerating action of the platelet extract and reduce or eliminate the difference between the coagulability of the plasma in glass and lusteroid tubes. In the dilution used (1-2,000), brain extract clots plasma in approximately the same time in glass and



TABLE 6

*Effect of various thromboplastic agents on the clotting time\* (in glass and lusteroid tubes) of undiluted and diluted hemophilic plasma and of normal plasma exposed to asbestos fibers (10 mgm./1 ml. plasma)*

THROMBOPL. AGENT	CONC. OF PLASMA	PERIOD OF INCUB.	HEMOPH. PLASMA		NORMAL PLASMA									
					Duration (mins.) of contact with asbestos									
					0		15		30		60		120	
			Gl.	Lu.	Gl.	Lu.	Gl.	Lu.	Gl.	Lu.	Gl.	Lu.	Gl.	Lu.
Cephalin	%	mins.												
	100	0	189	653	104	187	62	85	44	47	40	40	36	36
	100	20	327	4065	121	374	83	159	54	62	56	56	48	45
	20	0	210	320	68	134							48	45
Platelet extract	20	20	305	462	86	185							67	69
	100	0	652*	1605	126	441							94	94
	100	20	690	3060	102	396							80	87
	20	0	470	597	115	256							172	186
Dilute brain extract	20	20	380	646	98	244							130	130
	100	0	80	91	78	86	59	59	45	45	40	40	38	38
	20	0	68	72	67	77					56	56	63	63
Viper venom 1-500,000	100	0	68	68	70	70	70	70	74	71	68	69	81	81
	20	0	87	85	82	82					107	107	154	154

\* 0.3 cc. plasma, 0.1 cc. thromboplastic agent (0 or 20 min. incubation), 0.1 cc.  $\text{CaCl}_2$ .

All figures in the body of the table express clotting time in seconds, unless otherwise noted.

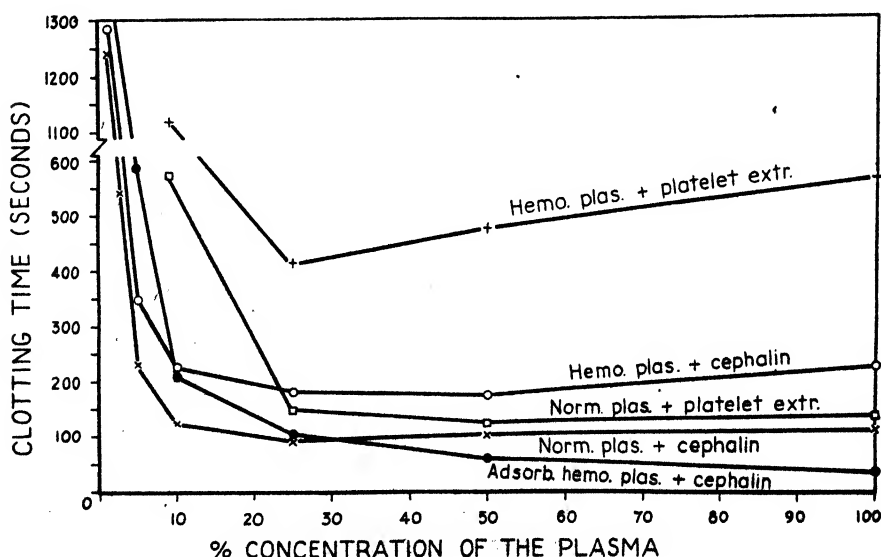


Fig. 2. Effect of dilution of the plasma on the clot accelerating action of cephalin and platelet extract. Adsorbed plasma: hemophilic plasma after contact with asbestos fibers for 2 hours at 20°C. (10 mgm. per 1 ml. plasma). Glass tubes employed.

lusteroid tubes and its clot accelerating action is, like cephalin, diminished by incubation (4) and enhanced by diluting the plasma or exposing it to adsorbents (table 6). The clotting time of plasma to which Russel Viper Venom is added is prolonged by dilution of the plasma, and not significantly shortened by exposure to adsorbents. The venom seems invulnerable to anticephalin (5) and clots plasma whether hemophilic, normal or adsorbed, in about the same time in glass or lusteroid tubes.

If dilution is continued under 5 per cent, it almost effaces the difference in the reaction of hemophilic and normal plasmas towards cephalin (fig. 2) and equalizes their prothrombin conversion rates (10). This fact in itself seems to support the viewpoint that in hemophilic plasma there is an excess of an inhibitor of coagulation rather than a deficiency of an accelerator.

COMMENT. The clot accelerating effect of contact with glass might result from the addition of something to the plasma, or by modification or removal of one or more factors already existing in the plasma. It is unlikely that glass tubes or infusorial earth filter candles release into the plasma substances which will alter its coagulability. It appears more likely that the enhanced coagulability of plasma in or after contact with glass, asbestos, kaolin, etc., results from an increase in the rate of conversion of prothrombin, as anticephalin is lost from the plasma. Surfaces like paraffin, collodion, lusteroid and acryloid preserve anticephalin activity much longer than glass, and thereby help to maintain the conversion of prothrombin by cephalin at a slow rate. Whitby and co-workers (11) attributed the clotting of plasma filtered through asbestos pads to some factor in crude asbestos which is removed by washing with 2N nitric acid. This should not necessarily imply that the factor is released into the plasma during contact. Treatment with acid alters the constitution of the chrysotile fiber (13), and changes its physical appearance and adsorptive properties. These changes perhaps are responsible for the lower efficacy of acid washed asbestos as an adsorbent. Hemophilic and normal plasmas in contact with crystalline (Gooch fiber) asbestos undergo changes similar to those observed when asbestos wool is used, but require contact for about twice as long a period of time.

A high content of silica enters into the composition of glass and of the more effective adsorbents employed. Plasma can be rendered hypercoagulable by contact with silicic acid powder itself. Perhaps the changes in the plasma after contact with glass and similar materials can be traced to the adsorptive properties of silica compounds in general.

Plasmas with an excess of anticephalin (e.g., hemophilic) appear to be better protected not only from activation by cephalin but also from the effect of contact with glass. Excessive anticephalin activity seems to account for the prolonged coagulation time of hemophilic blood in glass tubes and its even more delayed coagulation in paraffin coated tubes. In contrast, blood from normal individuals after an acute hemorrhage clots faster than normal, and at about the same rate, in the two types of tube. Plasma from such individuals has a low anticephalin activity (14) and resembles that after contact with a small amount of asbestos for a short period of time.

## SUMMARY

The coagulation of plasma proceeds at a slower rate in paraffin, collodion, "lusteroid" or "acryloid" tubes than in glass ones. Differences between the coagulability of a plasma in glass and in the other tubes generally parallel its degree of anticephalin activity. Dilution, or contact of the plasma with certain adsorbents, tends to equalize the behavior of the plasma in all tubes, to reduce anticephalin activity, and may eliminate or decrease the difference in coagulability and reaction toward cephalin, between normal and hemophilic plasmas.

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# HEART SIZE IN SHOCK PRODUCED BY VENOUS OCCLUSION OF THE HIND LIMBS OF THE DOG<sup>1</sup>

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While it is now definitely established that a reduction in minute cardiac output and stroke volume is a constant and contributing factor of the shock syndrome, little investigation has been carried out to determine whether or not this is associated with a reduction in heart size. It has been established that a reduction in venous pressure, which is part of the shock picture, will lead to a reduction in heart size (1-8) and such a reduction in heart size has been found to occur in shock when the heart is examined in the open chested animal (9-12). As far as we know there has been only one investigation on the heart size in shock as measured in the closed-chest animal with the x-ray, and in this study a decrease in heart size was reported (13). It is not clear from these studies to what extent the reduction in heart size is attributable to increased heart rate which, even in the absence of shock, leads to a decrease in heart size (1, 14). Furthermore, it is well known that anoxemia, due to the slowed circulation, complicates shock and this could obscure the effect of heart rate and reduction in venous returns since it is known that anoxemia leads to cardiac dilatation (15, 16, 17). It was considered important, therefore, to investigate changes in heart size using the x-ray in the intact closed-chest dog during various stages in the development of shock. In this study, an attempt was made to distinguish between the changes in heart size accompanying tachycardia from those attributable directly to shock. In some experiments atropine was administered during the course of the experiment in order to minimize heart rate changes.

**PROCEDURE.** Dogs anesthetized with nembutal (25 mgm./K. intraperitoneally) were used. The operative procedure for producing shock was that of injecting lampblack into the external iliac veins of both hind limbs (18). In the atropinized dogs, 0.8 mgm. atropine was administered simultaneously with the anesthesia. Venous pressure was measured in the right external jugular or brachial vein by means of a single limb manometer filled with saline. Zero levels were obtained postmortem from the center of the right auricle. Arterial blood pressure was obtained directly from the femoral artery by means of the Hamilton needle manometer (19). Heart rate was determined by pulse count at the time of x-ray as well as from the blood pressure records.

X-rays were obtained in the x-ray department,<sup>2</sup> the tube being fixed at 36 inches from the chest of the dog, the exposure time being  $\frac{1}{20}$  sec., using a current

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of 100 milliamperes. Fifty-four kilovolts were used for the anteroposterior position and 57 for the lateral position. Before taking the x-ray films, the chests of long haired dogs were clipped for better contrast and to make the bony landmarks more easily identifiable.

Films were taken at the height of inspiration, except when studying the effects of respiration. For anteroposterior exposures the dogs were placed supine on an animal board, the body carefully aligned so that the vertebral column was straight on a center line, and the shoulders were fixed by two upright rods lateral to the neck, the fore and hind limbs being extended. The x-ray tube was centered on the level of the fourth intercostal space in the mid-sternal line. For the left lateral exposure, the dog was placed on its left side, with the forelimbs extended cephalad, the hind limbs caudad, and the back aligned along a straight line. The positions of the film and x-ray tube were not altered, the dog being simply turned on its side.

The heart shadow and chest silhouette of x-ray films so taken were retraced on translucent paper over a viewing box and the area of the heart and chest were measured in square centimeters with a planimeter.

All determinations and x-ray films were taken before, soon after and later during the course of shock after the bilateral venous occlusion of the hind-limbs had been performed. The procedure of this operation has already been described (18). All dogs were autopsied.

**RESULTS.** *a. Controls.* Several types of controls were used to determine the accuracy of the x-ray measurements.

The first was to eliminate the error involved in retracing the shadows and measuring the area by planimeter. In several cases the tracings made by several individuals were used to check biases. Nineteen films were retraced twice and the pairs of tracings measured several times. The variation in measuring the area of the anteroposterior heart shadow ranged from 1.5 to 5.8 per cent, averaging 2.8 per cent. The range for the lateral heart shadow was from 0.3 to 3.7 per cent, averaging 2.2 per cent. The measurements of the chest cavity size, in both views varied from 0 to 8.1 per cent, averaging 1.5 per cent. The error involved above was primarily an error of retracing the outlines since the error in planimetry is less than 1 per cent as shown by multiple trials. In 19 instances two anteroposterior films in succession were taken, all in inspiration, and the difference in the cardiac area ranged from 0 to 11.3 per cent, averaging 4.7 per cent. In these films the differences in chest size varied from 0.5 per cent to 9.3, averaging 3.2 per cent. These variations were greater than in retracing the same film and indicate that the increase was due to differences in phase of respiration, to spontaneous fluctuations in heart size, or to shifts in its position.

In 11 cases the effect on heart size of the phase of respiration at which the film was exposed was measured in anteroposterior films. In 5 cases the heart size during expiration was smaller than in inspiration by from 1.1 to 11.5 per cent, averaging 5.8 per cent. In 6 cases the heart size during expiration was larger than in inspiration by 0.2 to 10.7 per cent, averaging 3.8 per cent. Thus the range of change in heart size in respiration varied from an increase during

expiration of 10.7 per cent to a decrease of 11.5 per cent; the averages ranging from +3.8 to -5.8 per cent. The direction of change was not consistent, therefore, and was often outside the error of retracing. Consequently, in the experiments on shock, great care was taken to take all exposures at the height of inspiration.

The error of placement of the animal was next studied. For this purpose, an unoperated anesthetized dog was used and four sets of anteroposterior and lateral films were taken. The first and second were taken in quick succession, the dog being removed from the animal board between sets of exposures. The third and fourth sets were taken in like manner, two hours later; the heart rate in the first set was 122 and in the second was 132 beats/min. The difference in the heart area of the two anteroposterior films was 6.8 per cent for the first set and 6.0 per cent for the second. The difference in the heart area of the two lateral films was 7.4 per cent for the first set and 6.0 per cent for the second. The difference in the chest size between pairs of film ranged from 8.6 and 7.9 per cent in the lateral view and 2.8 and 3.9 per cent in the anteroposterior view. The error introduced by this procedure included the errors of the previous controls (except variation in phase of respiration) and the error of proper placement. The latter apparently was not as great as the former since the differences found in the sets was not increased much over that in checking pairs of retracings, or in taking two films in succession.

In one instance the effect of a marked change in heart rate on an unoperated anesthetized animal was noted. This dog was ergotamized (0.5 mgm. ergotamine tartrate intravenously) and the first films were taken when the heart rate was 87 beats/min. Then 0.9 mgm. atropine sulfate was administered intravenously and when the heart rate increased to 144 beats/min. the second films were taken. This 57 beat per minute change in heart rate caused a decrease of 3.2 per cent in the heart shadow in the anteroposterior view and a 1.4 per cent decrease in the lateral view. These changes are well within the experimental error of the method of making measurements. These results are in accord with those of Meek (14) who found only slight decreases in heart size when the rate increased up to 110 beats/min. and only above 110 beats/min. was there a noticeable decline in heart size which became more and more pronounced with each increment of heart acceleration.

These control studies incline us to believe that in an analysis of the changes in shock, a significant result can be considered as one in which the changes in heart size go in a consistent direction. Changes over 10 per cent are definitely significant, changes between 5 and 10 per cent if consistent should be considered probably significant, and changes less than 5 per cent are probably within the experimental error.

*b. Shock experiments.* A total of 10 shock experiments were carried out and these are tabulated in figure 1. Heart size in some experiments was determined from the area of the heart in the anteroposterior position; in other experiments, it was determined by the product of the heart areas in the anteroposterior and lateral views. The results in all experiments were consistent and in the same

direction, and for the most part outside the range of experimental error. No specific difference was found in the animals which were atropinized and those not atropinized; nor was there any significant difference in the animals having no significant cardiac acceleration ( $+10$  beats/min.) and those with definite cardiac acceleration (up to 50 beats/min.). In all, there was a definite decrease in heart size which was greatest immediately after operation and lessening progressively in the postoperative period. No clear correlation could be made between the amount of decrease in heart size and survival time. In some in-

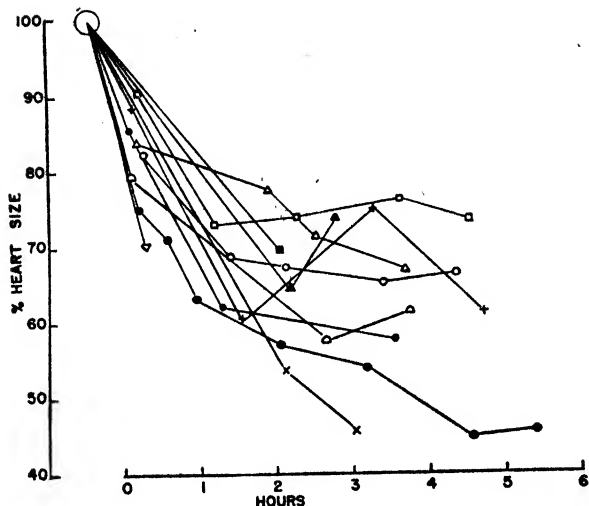


Fig. 1

Fig. 1. Data on change in heart size in 10 experiments following bilateral venous occlusion of the hind limbs of the dogs. Ordinates give per cent change in size, 100 per cent being the size of the heart in the teleoroentgenogram in the control period before operation was begun. Abscissae give time post-operatively in hours. In 5 dogs, heart rate changes were minimized by atropinization (viz., those experiments labelled by open triangles, open squares, solid circles and circles with crosses in them). Discussed in text.

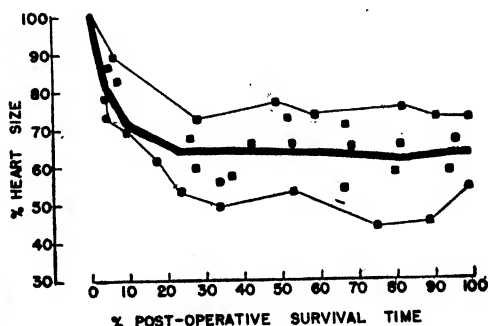


Fig. 2

Fig. 2. Graph depicting the effect on x-ray heart size following bilateral venous occlusion of the hind limbs of the dog. Ordinates as in figure 1. Abscissae give time post-operatively as per cent of total post-operative survival time, total post-operative survival time being considered as 100 per cent. Heavy line shows the mean of the values, the thin lines delineate the scatter of the individual values shown as points on the graph. All the values of figure 1 are included. Discussed in text.

stances, in those dogs surviving longest, the heart size became stationary after the first or second hour, and in several there was a definite small terminal increase in heart size.

The great variability in survival time suggested that the results could be more clearly depicted by plotting the heart sizes against a time scale in which the total postoperative survival time regardless of its duration is considered 100 per cent and the time at which the reading was made measured as a percentage of the entire survival time. The results of this recalculation are shown

in figure 2. A definite and consistent pattern of change in heart size is revealed by the mean values and the scattered points. The heart decreases in all instances in curvilinear fashion, steepest in the first 5 per cent of the postoperative survival period, and this decrease becomes gradually less and less until at the end of the first 25 per cent of the postoperative survival period, the change is insignificant. In fact in the last 20 per cent of the postoperative survival period there is a tendency for heart size to increase slightly.

DISCUSSION. These results show that shock produced by bilateral venous occlusion definitely leads to a decrease in heart size. This decrease in heart size occurs even in atropinized animals and when heart rate acceleration is minimal or absent. It is apparently due to the loss of circulating blood volume into the operated limbs, a process which initiates the vicious cycle of the shock syndrome. The heart shares in this diminution of size. The decrease in size of the heart is an early phenomenon occurring during the period when shock is beginning. This would lend support to the view that a cardiogenic factor comes into operation early to contribute to the slowing of the rate of circulation. This cardiogenic factor is not a primary one but a secondary one resulting from loss of blood volume which decreases heart size and the minute output that it pumps.

The lesser decrease in heart size later is in part presumably due to the lessened rate of loss of fluid into the occluded extremities as well as the re-entry of fluid into the vascular system from the extravascular compartments. It also is attributable in part to the ill-effects of the developing shock tending to impair the condition of the heart and thereby leading to loss of tone. Among the ill-effects that might operate in this way, the most obvious is ischemia, since coronary flow should share in the decline in the total systemic flow, particularly as the blood pressure falls. Doubtless other, and at present less clearly defined, effects on the metabolism of the heart operate to lead to loss of tone. Such a loss of cardiac tone, tending to increase heart size, counteracts in a progressively greater and greater degree the effect of decreasing circulating blood volume, until toward the end it actually dominates slightly.

If this interpretation is correct, the cardiac contribution to the progression and ultimate irreversibility of shock is a two-fold one. The first is the result of loss of circulating blood volume which in the type of experiment performed in this study initiates the state of shock. The second is an impairment of the tone of the heart and presumably also its contractile power as a result of the slowed circulation. The latter would contribute to embarrass the circulation further. There remains little doubt that such observations on changes in heart size are compatible with the view that vicious cycles involving the heart itself contribute to the progression of shock and to its irreversibility.

#### SUMMARY

Changes in heart size following the production of shock by bilateral venous occlusion of the hind limbs of the dog were studied by means of x-ray. X-ray films of the heart in the anteroposterior and left lateral positions were taken, retraced, and the shadow area determined by planimetry. In some experiments, heart rate was controlled by atropinization.



Control studies showed that a change of heart size of 5 per cent, if uniform and consistent, was probably significant, and a 10 per cent change was unequivocal.

The operative procedure employed led to a consistent decline in heart size greatest in the first hour. This decline was attributed to the loss of circulating blood volume as shock developed, the rate of loss lessening as the experiment progressed.

The rate of decline in heart size lessened after the first quarter of the post-operative survival and later the heart size did not change or, even, increased slightly. It is suggested that these later changes may be due, in part at least, to the development of myocardial ischemia, as a result of the slowed rate of blood flow, shared by the coronary circuit, leading to a loss of cardiac tone.

We are indebted to Mr. Gerald Graham for his assistance in the execution of these studies.

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# THE EFFECTS OF INTRACISTERNAL INJECTION OF SODIUM BROMIDE UPON THE BLOOD-SPINAL FLUID BARRIER

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In a previous study data were reported which indicate that the value of the distribution ratio of bromide ion between serum and cerebrospinal fluid varies inversely as the concentration of bromide ion in the serum (1). At low serum bromide levels in dogs, the ratio  $\frac{[\text{Br}^-]_s}{[\text{Br}^-]_{\text{sp. fl.}}}$  approaches 2; at very high serum bromide levels the ratio approaches unity. The significance of these values is entirely different. The higher value must be interpreted as indicating the existence of one or more factors which prevent the free diffusion of bromide ion from the serum into the spinal fluid or, as usually stated, as indicating the existence of a "barrier" to the passage of bromide ion into the cerebrospinal fluid. On the other hand, a distribution ratio at or near unity suggests that a process of filtration is operative and, by implication, that no barrier to the free passage of the substance in question exists.

The observation that the ability of bromide ion to gain access to the cerebrospinal fluid may be altered experimentally seemed sufficiently important, especially in view of our imperfect knowledge regarding the exact mechanism of the formation of the cerebrospinal fluid, to warrant further investigation of this finding utilizing a different approach. In addition, experiments were performed to determine whether the distribution of other ions normally present in the body fluids is affected by the conditions which affect the bromide distribution.

The general procedure followed in these experiments was designed to produce initial local increases in the bromide concentration of the cerebrospinal fluid while maintaining the bromide concentration of the serum at a level at which previous experiments had shown it possible to demonstrate the presence of the "bromide barrier." To attain these conditions, hypertonic solutions of sodium bromide were administered intravenously to a series of dogs. After an equilibration period of 24 hours, and after withdrawing a sample of blood and of spinal fluid for bromide analysis, a hypertonic solution of sodium bromide (0.6 gram NaBr in 3 cc. of water) was injected intracisternally to produce a local increase in the bromide concentration of the spinal fluid. Thereafter, and without any further bromide administration unless otherwise stated, samples of blood and spinal fluid were withdrawn periodically for analysis. Needle puncture of the cistern was done under ether anesthesia.

**METHODS.** This study involved the determination of bromide, total halide, water, sodium and potassium, in serum and spinal fluid. The methods employed for bromide, total halide and water have been described previously (2). Sodium was determined according to the method of Butler and Tuthill (3). For the

determination of potassium the method of Shohl and Bennett (4) was used employing two minor technical modifications. First, because platinum crucibles were not available, silicon crucibles were used; second, instead of making asbestos filters in small funnels as recommended, the filters were formed in small thistle tubes. These were fitted at the top with one hole rubber stoppers through which was inserted one end of a small inverted "U" tube. The other end of the U tube was dipped into the solution to be filtered, while the stem of the thistle tube was

TABLE 1

*Bromide distribution ratio following intracisternal administration of sodium bromide*

SAMPLE	$\frac{[Br]_{mM}}{KGM. H_2O}$	$\frac{H_2O \text{ GRAM}}{KGM.}$	$\frac{[Br]_s}{[Br]_{sp. fl.}}$	TIME
Dog 1—wt. 16.7 kgm. 20 grams NaBr intravenously. Initial serum and spinal fluid (So and Sp.fl.) withdrawn 24 hrs. later after which 0.6 gram NaBr in 3 cc. H <sub>2</sub> O injected intracisternally.				
				hrs.
Serum <sub>0</sub> .....	24.4	920.0	1.33	0
Serum <sub>1</sub> .....	24.8	912.0	1.08	26
Serum <sub>2</sub> .....	21.4	911.0	0.98	46
Serum <sub>3</sub> .....	22.0	912.0	1.08	72
Serum <sub>4</sub> .....	22.0	911.0	1.08	96
Spinal fluid <sub>0</sub> .....	19.6	990.0		
Spinal fluid <sub>1</sub> .....	24.8	988.0		
Spinal fluid <sub>2</sub> .....	24.0	995.0		
Spinal fluid <sub>3</sub> .....	22.0	994.0		
Spinal fluid <sub>4</sub> .....	22.0	988.0		
Dog 2—male. Wt. 15 kgm. 20 grams NaBr intravenously. So and Sp. fl. 24 hrs. later after which 0.6 gram NaBr in 3 cc. H <sub>2</sub> O injected intracisternally.				
Serum <sub>0</sub> .....	32.3	928.0	1.21	0
Serum <sub>1</sub> .....	29.2	924.0	0.98	26
Serum <sub>2</sub> .....	28.8	925.0	1.04	52
Serum <sub>3</sub> .....	25.2	928.0	1.10	72
Serum <sub>4</sub> .....	27.3	923.0	1.12	96
Spinal fluid <sub>0</sub> .....	26.7	988.0		
Spinal fluid <sub>1</sub> .....	29.8	986.0		
Spinal fluid <sub>2</sub> .....	27.2	986.2		
Spinal fluid <sub>3</sub> .....	23.3	986.7		
Spinal fluid <sub>4</sub> .....	24.3	988.0		

applied to a suction flask as usual. This modification obviates the necessity of pouring the solution into the small filter funnel and thereby avoids the risk of drops of solution running down over the outside of the crucible in which the precipitation has taken place. Both the methods for sodium and potassium were checked initially against known solutions and were found to yield theoretical returns.

**RESULTS.** In table 1 are presented complete data obtained on two dogs in a

series of six treated similarly. Since all dogs in the series showed the same response to intracisternally administered sodium bromide, a detailed report upon the remaining dogs would serve no useful purpose.

It will be noted that the ratio  $\frac{[\text{Br}^-]_s}{[\text{Br}^-]_{\text{sp. fl.}}}$  remains depressed following the intracisternal administration of sodium bromide for as long as four days. The values obtained are more significant when considered in the light of our previous report which indicated that in the large series of dogs studied such low values were never obtained by alteration of the serum bromide level until the concentration of bromide in the serum reached a range from 70 to 90 millimols of bromide per liter of serum; and indeed even then values as low as 0.98 were not reached. At serum

TABLE 2

SAMPLE	$\frac{\text{Br mM}}{\text{KGM. H}_2\text{O}}$	$\frac{\text{H}_2\text{O GRAM}}{\text{KGM.}}$	$\frac{[\text{Br}]_s}{[\text{Br}]_{\text{SP. FL.}}}$	REMARKS
Dog 3—Female. Wt. 14 kgm. 20 grams NaBr intravenously. After So and Sp. fl. <sub>0</sub> were withdrawn 0.6 gram NaBr were injected intracisternally.				
Serum <sub>0</sub> .....	33.9	925.0	1.19	After initial NaBr by vein
Serum <sub>1</sub> .....	34.7	928.0	1.04	After intracisternal NaBr
Serum <sub>2</sub> .....	44.0	924.5	1.08	After second intravenous dose of NaBr (4.0 grams) 48 hours between samples
Sp. fl. <sub>0</sub> .....	28.4	991.0		
Sp. fl. <sub>1</sub> .....	33.5	990.6		
Sp. fl. <sub>2</sub> .....	41.0	986.0		
Dog 4—Female. Injected with 20 grams NaBr intravenously				
Serum <sub>0</sub> .....	38.6	921.0	1.16	After initial NaBr by vein
Serum <sub>1</sub> .....	40.2	916.0	1.06	After intracisternal NaBr
Serum <sub>2</sub> .....	40.4	920.0	1.05	After a second intravenous dose of NaBr (4 grams) 48 hours between samples
Sp. fl. <sub>0</sub> .....	33.0	984.0		
Sp. fl. <sub>1</sub> .....	37.7	985.0		
Sp. fl. <sub>2</sub> .....	38.5	987.0		

bromide levels such as maintained in these animals (10 to 30 mM/L.) one would expect the value of the ratio  $\frac{[\text{Br}^-]_s}{[\text{Br}^-]_{\text{sp. fl.}}}$  to fluctuate about an average of 1.25.

Reference to table 1 shows that the two dogs reported here fulfilled this expectation before sodium bromide was administered intracisternally.

These observations raised the interesting question as to what effect intravenous administration of sodium bromide would have upon the value of the ratio while, as the result of intracisternal injection of sodium bromide, the bromide barrier is depressed. In table 2 are reported experiments directed at this question.

Apparently artificial restoration of the normal ratio (1.19 in dog 3 and 1.16 in dog 4) which must have occurred at some time between the second intra-

venous injection of NaBr (in hypertonic solution) and the attainment of the previous low ratio does not result in the maintenance of the ratio. The alteration of the ratio by intracisternal injection of sodium bromide therefore seems permanent while the effects persist.

In table 3 is shown a summary of results of experiments conducted to determine the time at which equilibrium is reached following intracisternal injection of sodium bromide. These data indicate that equilibrium is attained slowly, from 6 to 24 hours being required. These findings are consistent with observations reported previously that equilibrium between serum and spinal fluid following intravenous injection of sodium bromide is also slow (1, 5). The equilibrium attained here may be more apparent than real. It is possible that the low values of the ratio obtained by intracisternal injection of sodium bromide represent a stage of arrest in the re-establishment of equilibrium. From this point of view this apparent equilibrium is attained within 24 hours, is maintained for several days, and is followed by a more gradual reversion, as recovery occurs, to a truer type of equilibrium which is a function of the serum bromide level and perhaps other factors.

TABLE 3

*Rate of attainment of equilibrium following intracisternal administration of sodium bromide*

DOG NO.	$\frac{[\text{Br}]_s}{[\text{Br}]_{\text{SP. FL.}}}$	TIME
		<i>hrs.</i>
5	0.91	6.25
6	0.99	6.25
7	0.72	9.5
8	0.90	8.0
	0.98	25.0

In table 4 are presented the results of analysis of serum and spinal fluid for sodium, potassium and total halide following the intracisternal injection of sodium bromide. It was not possible, because of limited samples of spinal fluid, to analyze these samples for bromide also. However, since these experiments were conducted in the same manner as those of table 1, and since the latter were so consistent in showing a lowering of the barrier to the passage of bromide ion, it is assumed that the same lowering occurred in these experiments. The terms "before" and "after" in the table indicate "before" and "after" intracisternal injection of NaBr.

These data, we feel, indicate that the intracisternal injection of sodium bromide produces effects in the spinal fluid upon distribution ratios other than bromide. It is to be noted that although the distribution ratio of bromide decreases under the conditions of the above experiments the halide ratio ( $\text{Br} + \text{Cl}$ ) increases, suggesting an over-compensation by chloride to the increased amount of bromide in the spinal fluid. The potassium changes are interesting. The distribution ratio of potassium consistently decreased in all experiments although in dog 11 the decrease was more at the expense of a decrease in serum potassium than an

elevation of spinal fluid potassium. As far as we were able to ascertain qualitatively this change was not due to alterations in protein content of the spinal fluid. Most variable were the changes in the distribution of sodium ion. This ratio increased in three dogs, remained unchanged in one dog and decreased in one dog, but in no case did the value vary significantly from unity.

DISCUSSION. The action of hypertonic solutions of sodium bromide injected intracisternally in amounts that have little or no demonstrable effect upon the serum bromide level is identical in effect to large amounts of sodium bromide

TABLE 4

*Distribution ratio of sodium, potassium and total halide before and after intracisternal administration of sodium bromide*

DOG	SAMPLE	Na	K	HALIDE	$\frac{Na_s}{Na_{SP. FL.}}$	$\frac{K_s}{K_{SP. FL.}}$	$\frac{HALIDE_s}{HALIDE_{SP. FL.}}$
					<i>mM/Kgm. of water</i>		
9	Serum before	155.5	3.65		0.99	1.32	
	Sp. fl. before	157.2	2.76				
	Serum after	157.0	3.61		1.03	1.0	
	Sp. fl. after	152.0	3.60				
10	Serum before	151.8	3.41	114.6	1.00	1.32	0.91
	Sp. fl. before	150.7	2.57	126.5			
	Serum after	156.5	3.90	116.8	1.04	1.20	0.96
	Sp. fl. after	150.2	3.24	121.2			
11	Serum before	161.5	3.89	118.4	1.04	1.47	0.93
	Sp. fl. before	155.0	2.65	127.5			
	Serum after	152.0	3.45	117.0	1.02	1.32	0.96
	Sp. fl. after	149.0	2.61	121.0			
12	Serum before	157.5	4.99	117.2	1.04	2.15	0.92
	Sp. fl. before	152.0	2.32	127.1			
	Serum after	156.7	4.57	118.0	1.04	1.29	0.95
	Sp. fl. after	150.5	3.54	123.5			
13	Serum before	154.0	2.97	118.8	1.01	1.28	0.93
	Sp. fl. before	152.3	2.32	127.5			
	Serum after	158.0	2.79	124.2	1.03	0.95	0.98
	Sp. fl. after	152.7	2.94	126.0			

injected intravenously in that both procedures result in a lowering of the bromide barrier between the serum and cerebrospinal fluid. The mechanism of action in the two cases need not be the same. In any event we are not able to state how these solutions produce their effect.

In the case of intracisternal injections the effect could be due to the  $Na^+$ , the  $Br^-$ , the effect of the osmolar concentration of the solution, or any combination of these. That bromide ion is not essential is shown by the fact that the same lowering of the barrier to bromide ion can be caused by intracisternal injections of hypertonic NaCl (0.4 gram NaCl in 3 cc.  $H_2O$ ). Neither is the effect

due simply to intracisternal injection, for introduction of distilled water (5 cc.) produces no alteration in the distribution ratio of bromide ion. Certain observations suggest that the action of hypertonic NaBr need not be considered as taking place directly upon the membranes across which the cerebrospinal fluid is formed but may be due to a more remote action. In some animals muscular twitchings about the face and neck were noted during or immediately after the intracisternal injection. Sometimes an immediate cessation of respiration occurred and artificial respiration for a few moments was necessary after which normal respiration resumed. A blood pressure record taken on one animal revealed an immediate and marked rise in blood pressure and evidence of vagal stimulation which however may have been a secondary effect. The blood pressure and respiratory effects were on the whole strikingly similar to those seen in animals following experimental increases in intracranial pressure. However, the abruptness of the onset of these effects as well as the magnitude of the effects, precludes the assumption that the hypertonic solution caused an ingress of fluid to the brain and a consequent elevation of intracranial pressure. All of these effects were over within 10 to 15 minutes.

Until more is known about the mechanisms by which the spinal fluid is formed it is perhaps futile to speculate at length about factors which appear to disturb the normal mechanism. Nevertheless a working hypothesis is useful. If we follow Weed (6) in his belief that the spinal fluid has dual source, one contribution coming from the choroid plexus and another contribution from the capillaries of the brain via the pericapillary spaces we can visualize a simple explanation of the data obtained.

The data indicate that insofar as total halide, bromide and potassium are concerned the spinal fluid after intracisternal injection more closely resembles an ultrafiltrate of serum than before the injection. The sodium ratio is consistent with an ultrafiltrate before and after injection. To explain these findings we need only assume that the intracisternal injections either depress (by direct action and/or reflexly) the choroid plexus or similarly increase the amount of filtrate normally contributed by the capillaries of the brain.

#### CONCLUSION

Evidence is presented that intracisternal injections of sodium bromide in amounts too small significantly to alter the serum bromide level lower the barrier to the passage of bromide ion into the spinal fluid. The distribution ratio of potassium and chloride ion is affected also by this procedure. Analysis of the spinal fluid after intracisternal injection reveals that the fluid more closely resembles an ultrafiltrate of serum than before the injection.

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# INTRAMUSCULAR PRESSURE CHANGES IN SHOCK<sup>1</sup>

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According to Henderson and his co-workers (1-4) and Gunther and collaborators (5-7), the intramuscular pressure of the body muscles is an important factor in shock since it is considered by them to be vitally concerned in maintaining the peripheral circulation, venous pressure, volume of venous flow and cardiac filling. Thus, in Henderson's terminology, intramuscular pressure is "muscle tonus" and shock is "hyp-tonia." Despite the recent studies of Gunther *et al.* which support and extend Henderson's thesis, little interest has been evinced in this aspect of the problem by students of shock.

The object of the present experiments was to study intramuscular pressure changes in dogs shocked by a variety of procedures. Similar studies have been made on patients undergoing major operations, prolonged anesthesia and recovery from infections (1-7). However, so far as the writers are aware, reports have not been made on animals shocked under rigidly controlled conditions in which the shock terminated fatally.

Dogs which had been maintained under laboratory conditions for periods ranging from 7 to 14 days were employed as experimental animals. Mean arterial pressure was determined by needle puncture of the femoral artery (8) while the dogs were under pentobarbital sodium anesthesia. For measurement of intramuscular pressure in mm H<sub>2</sub>O, the instrument devised by Henderson (3) and modified by Kerr and Scott (9) was used. Gunther and Henstell (10) have further modified the apparatus so that it is possible to make simultaneous measurements of both intramuscular and venous pressures. The validity of the method utilized by Henderson has been experimentally checked by Hellebrandt, Criger and Kelso (11).

The medi-triceps muscle of both the fore limbs was used, with the dog lying upon its side, the limb extended and supported at right angles to the body. The IMP (intramuscular pressure) recorded in the tables represents an average of not less than six separate readings for each fore leg. A control study of 15 normal animals under pentobarbital sodium anesthesia, and involving several hundred determinations, gave readings of surprising uniformity (fig. 1).

1. *The intramuscular pressure of control dogs anesthetized with pentobarbital sodium.* An attempt was made to study IMP in non-anesthetized animals trained to lie quietly upon the table. Although a few of the dogs responded to training, the task proved so time-consuming we resorted to the use of anesthesia. The

<sup>1</sup> We are indebted to the Ciba Pharmaceutical Products Company of Summit, New Jersey, for providing funds to defray a part of the expenses of this investigation.

<sup>2</sup> Guest investigator on leave from the Ciba Pharmaceutical Products Company.



mechanism by which intramuscular pressure is altered is said to be quite sensitive to certain types of narcosis. Gunther (5) reported that ether and spinal anesthesia in man decrease IMP, and Cortell and Gellhorn (12) using cats, observed that chloralose prevented the rise in IMP which follows moderate bleeding. However, it has been our experience that pentobarbital sodium given intravenously in doses of 30 mgm. per kilogram of body weight, is satisfactory for studies of IMP. This proved to be the case even when the anesthesia was prolonged for 12 hour periods by repeated injections of the drug.

The effect of anesthesia was studied on 15 dogs, of which 11 were subjected to anesthesia of short duration, i.e., 2 to 5 hours, and 4 to anesthesia lasting 12 hours. The data presented in figure 1 failed to reveal any appreciable difference in IMP between the dogs anesthetized for either brief or long intervals. It is probable that owing to the anesthesia, both initial and final readings of IMP fall below the lower range of normal. This is partially offset, however, by the uniformity of the conditions under which the readings were obtained. Hellebrandt, Crigler and Kelso (11) report that in human subjects, the IMP in the relaxed muscle is rela-

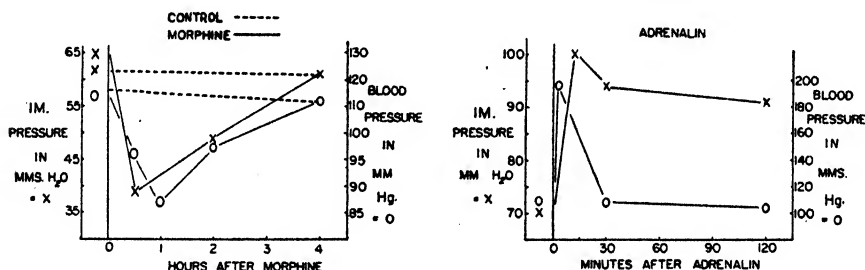


Fig. 1. The effect of (1) pentobarbital sodium anesthesia (2-12 hrs.); (2) morphine plus pentobarbital sodium anesthesia, and (3) adrenalin, upon intramuscular and blood pressure.

tively constant. In any case, since it was imperative to employ deep anesthesia in the dogs subjected to shock inducing procedures, there seemed to be no simple way of avoiding sources of error due to this cause.

2. *The effect of adrenalin and morphine upon the intramuscular pressure of anesthetized dogs.* The uniformity of the IMP readings of the anesthetized dogs led us to test their validity by employing drugs which have been reported to elevate or lower IMP in an unmistakable manner. Adrenalin and morphine were used. Beiglbock and Steinlechner (13) showed that adrenalin increases IMP. This has been confirmed by Kiely, Hamilton and Gellhorn (14), and the present experiments afford further supporting evidence. An intravenous injection of 5  $\gamma$ /kgm. of adrenalin in the medium size, anesthetized dog, markedly raised both blood pressure and IMP. However, the IMP remained high, long after the blood pressure returned to normal levels. On the other hand, morphine (30 mgm.) given subcutaneously, and followed 30 minutes later by intravenous administration of 20 mgm./kgm. of pentobarbital sodium, decreased both IMP and mean arterial pressure, the effect persisting for several hours (fig. 1). The fact

that it was possible to raise or lower the IMP from the average control readings at will by drug action, and that the control readings were always attained after the effects of the drugs had worn off, convinced us that the control IMP readings were reasonably accurate.

3. *Intramuscular pressure changes in shock induced by release of limb tourniquets.* The application of tight tourniquets placed high on both hind legs of anesthetized dogs, and released after 5 hours, almost invariably results in fatal shock. Thus, of 25 untreated control dogs, all but one died in shock following release of the constrictions (15). This type of shock is characterized by marked loss of plasma through the capillaries of the injured legs, resulting in a 49 per cent reduction in plasma volume, extreme hemoconcentration and drastic decline in arterial pressure. During the 5 hour period previous to removal of the constrictions, the animals exhibit little change from normal with respect to blood concentration, plasma loss or arterial pressure. Since release of leg tourniquets led to a 96 per

TABLE 1

*Intramuscular pressure changes in shock induced by release of limb tourniquets*

DOG NO.	WEIGHT KGM.	IMP MM. H <sub>2</sub> O INITIAL	B.P. MM. Hg INITIAL	TOURNIQUETS 4 HOURS		TOURNIQUETS RELEASED		3-5 HOURS		6-10 HOURS		IMP DECREASE FROM INITIAL PER CENT
				IMP	B.P.	IMP	B.P.	IMP	B.P.	IMP	B.P.	
1	7.2	54	112	40	108	35	104	29	72	24	60	56
2	6.8	64	105	59		57	102	33	56	22	47	66
3	15.0	58	102	53		52	119	49	108	37	81	36
4	7.6	61	129	59	120	53	116	37	88	30	70	51
5	17.0	61	121	54	127	54	110	31	61			49
6	8.0	56	119	43		50	120	24	81	24	74	57
Ave.	10.3	59	115	51	118	50	112	34	78	27	66	52

cent mortality, this procedure was considered an excellent one for studying IMP changes in shock due primarily to plasma loss into the area of injury.

The essential data are given in table 1 and show that IMP decreases with the development of shock symptoms. Several hours before death, the IMP had declined to an average of 52 per cent of the initial readings. This was the most striking fall in IMP we encountered in any type of shock studied. In two dogs (nos. 1 and 6) a fall in IMP occurred during the period shortly before release of the limb constrictions. Only one dog of the six used was studied to the end, i.e., no. 2; the remaining five survived for several hours after the last readings were obtained and died some time during the night.

4. *Intramuscular pressure changes in shock induced by application of a limb press.* Duncan and Blalock (16) devised a compression apparatus which when applied at 500 pounds' pressure to one hind limb of the anesthetized dog, and left in place for 5 hours, induced fatal shock within a short time after release. Shock resulting from this procedure is accompanied by marked plasma loss into the injured leg, severe hemoconcentration and low arterial pressure. In our experi-

ments, fatal shock was induced in 25 of 26 untreated control dogs following release of the compression. The plasma volume declined 48 per cent and autopsy indicated that most, if not all of the plasma volume reduction, could be accounted for by plasma loss into the injured extremity (17).

In the experiments mentioned, the leg press was adjusted to 750 pounds' pressure and left on the limb for 7 hours. The dogs were given 30 mgm. of morphine subcutaneously followed within 30 minutes by an intravenous injection of 20 mgm. of pentobarbital sodium per kilogram of body weight. However, for obvious reasons, the morphine was omitted in the experiments involving IMP, and 30 instead of 20 mgm. per kilogram of pentobarbital sodium were given intravenously. In these experiments the shocked dogs survived longer than usual owing to the fact that morphine was not given. As a result terminal IMP readings were not obtained except in one case (no. 2, table 2). Most of the dogs died

TABLE 2

*Intramuscular pressure changes in shock induced by application of a leg press*

DOG NO.	WEIGHT KGM.	IMP MM. H <sub>2</sub> O INITIAL	B.P. MM. Hg INITIAL	PRESS RELEASED		3-5 HOURS		6-10 HOURS		IMP DECREASE FROM INITIAL PER CENT
				IMP	B.P.	IMP	B.P.	IMP	B.P.	
1	12.9	70	118	56	140	46	89	37	54	47
2	7.1	62	109	55	113	38*	49			39
3	12.7	66	115	59	142	48	65	41	60	38
4	9.3	71	101	63	122	49	80	47	66	34†
5	9.6	67	131	63	140	47	82	48	74	28
6	12.0	69	120	62	152	54	98	41	62	41
7	12.5	71	129	63	135	63	105	40	48	44
8	14.5	67	131	62	130	44	72	33	68	51
Ave.	11.3	68	119	60	134	49	80	41	62	40

\* Dog died one hour after last reading. Terminal readings not obtained on remaining 7 animals.

† Animal recovered. See text.

during the night at a time when no one was available to take the readings. Despite the lack of terminal data, the IMP declined as shock developed and decreases in IMP ranging 28 to 51 per cent of the normal occurred. The average decline for the 8 dogs studied was 40 per cent. There was a slight decrease in IMP during the 7 hour interval the press was on the leg. One animal (no. 4, table 2) recovered from shock. Although not recorded in the table, the IMP of this animal slowly rose as the arterial pressure returned to the control level and shock symptoms disappeared.

5. *Intramuscular pressure changes in shock induced by muscle trauma.* Fatal shock is easily produced in deeply anesthetized dogs by several muscle trauma techniques. Probably the simplest and most efficient method is that reported by Gregersen in which 400 to 800 blows are distributed over all surfaces of each thigh, with a 200 gram rawhide mallet. The writers have employed this method in

studies concerned with the nervous factor in shock, and the efficacy of infusions of plasma, and the plasma substitutes gelatin and saline as prophylaxis against circulatory failure following injury (18). Muscle trauma of the type described led to fatal shock in 33 or 89 per cent of 37 anesthetized dogs. The shock is characterized by lack of hemoconcentration, loss of whole blood into the injured area, drastic fall in blood pressure and a decline in plasma volume of approximately 30 to 35 per cent. An important nervous factor appears to be present which contributes to the initiation of shock.

The pertinent data relating to changes in intramuscular pressure in shock induced by muscle trauma are shown in table 3. The IMP did not change immediately following trauma, despite lowering of blood pressure. During succeeding hours (6-10 after trauma) the decline was marked. The average change in IMP during shock from the initial readings was 49 per cent with a range of 39 to 63 per cent.

TABLE 3

*Changes in intramuscular pressure induced by leg muscle trauma*

DOG NO.	WEIGHT KGM.	IMP MM. H <sub>2</sub> O INITIAL	B.P. MM. Hg INITIAL	END OF TRAUMA		3-5 HOURS		6-10 HOURS		IMP DECREASE FROM INITIAL PER CENT
				IMP	B.P.	IMP	B.P.	IMP	B.P.	
1	8.6	69	138	62	67	49	84	25	70	63
2	9.3	66	111	61	65	64	95	40	42	39
3	8.2	72	124	70	69	44	51	29	38	60
4	11.7	72	107	65	67	50	43	37	35	49
5	11.9	66	123	67	51	50	56	39	30	41
6	12.0	66	103	60	58	47	41	40	34	40
Ave.	10.3	68	118	64	63	51	62	35	42	49

6. *Changes in intramuscular pressure induced by wound shock.* The procedure used for producing fatal wound shock was by gun shot injury to muscle masses of both hind legs of deeply anesthetized dogs. The procedure has been described in detail elsewhere (19) and need not be repeated here. Suffice it to state that 39 or 90.7 per cent of 43 anesthetized dogs not receiving treatment of any kind died in shock. The shock so induced is accompanied by a lack of hemoconcentration, sharp and sudden fall in arterial pressure, and loss of whole blood due to external and internal hemorrhage at the site of injury. The plasma volume declined approximately 44 per cent. Changes in IMP were followed from the time of injury to death, and are presented in table 4. The IMP declined as shock symptoms appeared, and shortly before death occurred the average fall in intramuscular pressure was 31 per cent. This figure represents the least change encountered in shock resulting from the four procedures used. It seems not improbable that the severe hemorrhage which follows this type of injury in most of the dogs may have masked the effect of muscle injury upon the IMP. It will be shown in the section which follows that severe hemorrhage, uncomplicated by

trauma, and not accompanied by shock, induced a sharp and rather prolonged rise in IMP. Thus in shock due to a combination of hemorrhage plus muscle injury, the intramuscular pressure readings would probably reflect the balance struck between the factor of hemorrhage which raises the IMP, and the factor of muscle trauma which decreases it.

The IMP is independent of sudden and drastic changes in blood pressure; this is shown in table 4. The arterial pressure fell immediately following injury and reached low levels with only a moderate recovery. However, IMP remained within the normal range and apparently did not reflect the fall in blood pressure. It is only after the lapse of several hours that a significant lowering of IMP occurred. Kiely *et al.* (14) have called attention to the independence of arterial pressure and IMP changes.

7. *Effect of hemorrhage and reinfusion of whole blood upon intramuscular pressure.* Some students of IMP changes in patients have stated that the intramuscular pressure of the muscles declines sharply following severe hemorrhage. We have not found this to be the case in nonshocked dogs subjected to blood loss represent-

TABLE 4  
*Intramuscular pressure changes in experimental wound shock*

DOG NO.	WEIGHT KGM.	IMP MM. H <sub>2</sub> O INITIAL	B.P. MM. Hg INITIAL	AFTER INJURY		3-5 HOURS		6-10 HOURS		IMP DECREASE FROM INITIAL PER CENT
				IMP	B.P.	IMP	B.P.	IMP	B.P.	
1	7.9	62	106	59	77	45	57	42	49	32
2	10.4	66	127	61	39	56	73	54	50	18
3	9.3	65	130	62	55	57	49	50	44	23
4	10.3	71	119	68	44	54	48			24
5	7.1	60	127	58	48	41	70	39	43	35
6	6.8	64	117	52	60	31	36			52
Ave.	8.6	65	121	60	54	47	56	46	47	31

ing 33 cc. per kilogram of body weight. On the contrary, the IMP increases in an unmistakable manner and attains levels beyond anything encountered in control animals. Our findings confirm those of Kiely, Hamilton and Gellhorn (14) and Gellhorn (20) who reported that hemorrhage in unanesthetized, decerebrate dogs leads to a sharp rise in IMP which returns to normal on reinfusion of whole blood.

In the six experiments reported here (table 5), the dogs were anesthetized, the femoral artery of the right leg cannulated and the blood allowed to flow freely until 33 cc. per kilogram had been removed. The blood pressure fell rapidly (table 5) to an average of 62 mm. Hg but spontaneously recovered to an average of 94 mm. Hg within a period ranging from 1 to 3 hours after bleeding. None of the animals exhibited signs of shock and recovery was complete when they were permitted to drink water *ad libitum* after anesthesia was discontinued.

The effects of hemorrhage upon IMP were unequivocal, for even before the bleeding was completed the intramuscular pressure rose rapidly and remained elevated for several hours. Return to normal tended to parallel the spontaneous

rise in blood pressure. The fact that IMP rose as the arterial pressure fell, immediately after hemorrhage, and declined as the blood pressure returned to normal might lead to the assumption that IMP and blood pressure were inversely related. However, the experiments of Kiely, Hamilton and Gellhorn (14) are contrary to such an assumption, for hemorrhage may lead to identical increases in IMP even when the fall in blood pressure is prevented by administration of pressor drugs which do not of themselves change the IMP.

The effect of reinfusing the blood removed is striking. The blood was drawn into a flask containing 0.5 cc. heparin, and maintained at 37°C. The infusion

TABLE 5

*Effect of hemorrhage upon intramuscular pressure of anesthetized dogs*

DOG NO.	WEIGHT KGM.	BEFORE BLEEDING		END OF BLEEDING		1-3 HOURS		5-7 HOURS		7-10 HOURS	
		IMP	B.P.	IMP	B.P.	IMP	B.P.	IMP	B.P.	IMP	B.P.
1	14.9	65	139	76	61	85	97	64	103	62	107
2	14.1	68	127	93	51	90	101	62	98		110
3	13.5	49	117	74	59	63	95	56	94		103
4	15.5	70	111	97	66	84	94	66	89	67	109
5	12.0	68	130	96	81	84	92	75	121	61	117
6	10.7	69	114	96	52	86	87	63	98	60	121
Ave.	13.5	65	123	89	62	82	94	64	100	63	111

TABLE 6

*Effect of hemorrhage and reinfusion of blood upon intramuscular pressure*

DOG NO.	WEIGHT KGM.	BEFORE BLEEDING		END OF BLEEDING		INFUSION					
		IMP	B.P.	IMP	B.P.	20 minutes		50 minutes		2-3 hours	
						IMP	B.P.	IMP	B.P.	IMP	B.P.
1	7.5	64	115	91	47	76	92	77	101	70	107
2	7.7	68	104	97	48	92	91	72	100	63	110
3	14.8	63	139	100	44	89	98	78	120	61	128
4	13.6	65	101	93	49	79	99	63	105	64	116
Ave.	10.9	65	115	95	47	84	95	73	107	65	115

was given by way of the jugular vein at a rate not exceeding 15 cc. per minute. The IMP which was in excess of 90 mm. H<sub>2</sub>O immediately after bleeding, fell to normal levels within 50 minutes after infusion and did not rise again thereafter (table 6).

If the elevation of IMP accompanying hemorrhage is regarded as reflecting an increase in tone of striated muscles, then it might be assumed that the somatic nervous system is somehow involved in the chain of reactions leading to vascular adjustment to severe hemorrhage. It has been demonstrated by others (14) that bilateral denervation of the carotid sinus area and bilateral vagotomy does not prevent the rise in IMP during bleeding.

## SUMMARY

1. Henderson's method was utilized for studying changes in IMP under the following experimental conditions: *a*, pentobarbital sodium anesthesia of long and short duration; *b*, before and after intravenous injection of adrenalin; *c*, following morphine plus pentobarbital sodium anesthesia; *d*, fatal shock induced by four different procedures; and *e*, sublethal hemorrhage without shock.

2. Pentobarbital sodium anesthesia of 12 hours' duration did not induce any greater change in IMP than anesthesia lasting 2 to 3 hours.

3. Adrenalin, administered by vein, raised both the blood pressure and IMP, but the latter remained elevated long after the blood pressure had returned to normal.

4. Morphine, followed by intravenously administered pentobarbital sodium, lowered both the arterial pressure and IMP for several hours.

5. Shock induced in deeply anesthetized dogs by *a*, release of limb tourniquets; *b*, application of a limb press; *c*, trauma to muscle masses of the hind legs; and *d*, gun shot injury was accompanied in all cases by marked fall in the IMP.

6. Sublethal hemorrhage, without shock representing 33 cc. per kilogram of body weight, caused a sharp rise in IMP which was maintained for several hours after spontaneous return of the blood pressure to normal.

7. Reinfusion of heparinized whole blood in the hemorrhaged dogs resulted in decline of the IMP to control levels.

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## TOURNIQUET SHOCK IN THE RABBIT<sup>1, 2</sup>

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Among the major obstacles in the study of shock have been the difficulty in reproducing the manoeuvres causing tissue injury, and the variability in the subsequently developing shock and the survival therefrom. Among the methods used for producing shock, that of occlusion of the circulation in certain parts of the body dates back to the experiments of Janeway and Jackson (1) since when several investigators have with variations followed the same technique in different animals. The present investigation deals with the production of shock resulting from tourniquet occlusion of the arterial and venous circulation of the hind limbs in the rabbit for varying intervals of time. Under the conditions, the survival time of the animals has proved to be remarkably predictable. In the studies of tourniquet shock on animals other than the rabbit, the survival times have when observed usually been very variable, though in 25 experiments by Swingle et al. (2) on the dog, a survival time of  $8 \pm 0.7$  hrs. was obtained after a five hour occlusion; while in 28 experiments on dogs by Mylon, Winternitz and de Suto-Nagy (3), 73 per cent of the animals died within 6 hrs., although the survival time varied from 1 to 48 hrs.

Of those who have thus far studied tourniquet shock in the rabbit, Allen (4) applied tourniquets to a single hind limb in two animals, both of which survived. Metzler (5) bound both hind legs of 4 rabbits in an Esmarch bandage for 2, 4, 6 and 10 hrs. respectively. Edema of the legs developed in 3 of the animals, but only the one that had been bound for 10 hrs. died. Paolucci (6) occluded both hind limbs for intervals varying from 1 to 5 hrs., but while a fall of blood pressure subsequently occurred, none of the animals died.

**METHODS.** Rabbits weighing from 2 to  $3\frac{1}{2}$  kilos were used. Light anesthesia was induced by intravenous nembutal (usually 15 mgm. per kilo), and was maintained by a concomitant administration of 1.5 to 2.0 grams per kilo of urethane subcutaneously.

In most of the experiments, the tourniquet consisted of a length of window-sash-cord, threaded through three or four small stab openings in the stretched skin to hold it in place, and then tightened about the upper portion of the thigh by means of a windlass. Later, thin walled  $\frac{1}{8}$  inch diameter rubber tubing was wound nine or ten times around the thigh, the coils constituting a constricting band of about 1 cm. in width, without breaking the skin. No difference in results was observed in the two methods. After varying occlusion periods the tourniquets were removed and the survival time noted, with intermittent observations of blood pressure, hematocrit and arterial and mixed venous blood  $O_2$  content. The

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<sup>2</sup> A preliminary report of these experiments was published in the Proceedings of the Federation of American Societies for Experimental Biology, March, 1944.



tourniquets were applied to both legs except when it was desired to estimate fluid loss.

**RESULTS.** In eleven control experiments in which the animals were maintained under anesthesia, the survival time averaged more than 24 hrs., in some cases the animals being killed during the second day. In 14 experiments the tourniquets were applied for 2 hrs., the survival time after release of the occlusion being  $3.8 \pm 0.6$  hrs. (table 1). The shortest survival was  $1\frac{1}{2}$  hrs., the longest  $8\frac{1}{2}$  hrs., while in 11 of the 14 experiments the range was between  $2\frac{1}{2}$  and 6 hrs. Increasing the period of occlusion from 2 hrs. to 5 hrs. brought about a concomitant

TABLE 1  
*Survival times of rabbits after tourniquet occlusion of both hind legs*

TYPE OF EXPERIMENT	NO. OF EXPTS.	SURVIVAL hrs.
(a) Anesthetic alone.....	11	More than 24
(b) Tourniquets applied for 2 hrs. room temp.....	14	$3.8 \pm 0.6^*$
(c) Tourniquets applied for 5 hrs. room temp.....	19	$1.7 \pm 0.4^*$
(d) Tourniquets applied 18-22 hrs.....	5	$0.2 \pm 0.08^*$
	8	$1.8 \pm 0.08^*$
(e) Tourniquets not released.....	24	$12.8 \pm 1.1$
Effect of temperature		
(f) Tourniquets applied for 5 hrs. legs in air bath at 37°C. during occlusion.....	15	$1.2 \pm 0.2^*$
(g) Tourniquets applied for 5 hrs. legs packed in ice during occlusion.....	14	$4.3 \pm 0.7^*$ 18, 24.5, >24, >24, and >48, respectively*
Effect of dehydration		
Water ad lib 7 days. Fasted (5 hr. occlusion).	6	$1.9 \pm 0.3^*$
No water for 7 days. Fasted (5 hr. occlusion).	7	$2.5 \pm 0.1^*$

\* Survival after release of tourniquet.

$\pm$  indicates standard mean deviation.

decrease in the survival time after release of the tourniquets, which averaged  $1.7 \pm 0.4$  hrs. in 19 experiments (table 1). The shortest survival time was 32 minutes, the longest 270 minutes. In 17 of the 19 experiments, the maximum range was between approximately 1 hr. and  $2\frac{1}{2}$  hrs.

We performed another series of experiments in which the tourniquets were not removed. The average survival time of this group of 24 animals was  $12.8 \pm 1.1$  hrs. We attribute the reduction in this survival time from that of the controls to absorption of toxic products from the region of the tourniquets, where crushing trauma had certainly occurred. In other words, it is really this group which is the best control for the occlusion experiments already described. In certain

animals the tourniquets were released after 18 to 22 hrs. In table 1 we have divided these into two groups, 5 in which the survival time after tourniquet release was  $0.2 \pm 0.08$  hrs.; and 8 in which the survival time was  $1.8 \pm 0.08$  hrs. The only difference between these two groups was that the former were performed in hot weather during the summer; they were our earliest experiments, and the later group, like all of our other experiments, were carried out in moderate room temperature ( $20-25^{\circ}\text{C}.$ ).

Of the occlusion and release experiments we regard the 5 hr. occlusion as the most predictable, and hence the most useful as a basis for evaluating therapy.

*Effect of temperature.* Allen (2) called attention to the fact that lowering the temperature of a ligated limb lessened the incidence of shock. The same result was obtained by Brooks and Duncan (7) in dogs. Our experiments confirm this for the rabbit. Thus (table 1) when the tourniqueted legs of rabbits were enclosed in a chamber at  $37^{\circ}\text{C}.$  for 5 hrs., the survival time averaged 1.2 hrs., while when packed in crushed ice the survival time in 14 experiments averaged  $4.3 \pm 0.7$  hrs., and in 5 additional experiments, which did not lend themselves to statistical analysis, the survival was from 18 to more than 48 hrs. When the tourniqueted limbs were chilled, ice tended to come in contact with regions directly above the tourniquet, and the body temperature as a whole fell, the average axillary temperature at the end of 5 hrs. being  $31.4^{\circ}\text{C}.$  compared with  $36.4^{\circ}\text{C}.$  in the animals whose limbs had been kept at  $37^{\circ}\text{C}.$  It is possible therefore to ascribe the longer survival to the lowered general body temperature. On the other hand, in these "cold" experiments the average axillary temperature after 5 hrs. of the 5 longest survivals was  $31.7^{\circ}\text{C}.$ , while that of the 5 shortest survivals (150 min.) was  $31.8^{\circ}\text{C}.$  This tends to favor the idea that the increased survival time was probably a function of the cooling of the tourniqueted limb itself.

*Blood  $\text{O}_2$  content and blood pressure.* The oxygen content of the mixed venous blood (right auricle) was determined in a number of control and "ligated" animals. The results were not unequivocal and in view of the small number of cases statistical analysis was unsafe. It can be said that there was a tendency for the oxygen content in most animals to fall as anesthesia was continued and that on the whole the fall was faster in the "ligated" animals.

The mean blood pressure showed three chief points of interest: 1. While the pressure tended to fall from the initial level in all animals, the fall was faster in the "ligated" ones, even before the tourniquet was removed, at which time the average mean pressure was about 80 mm. Hg. 2. While in the control animals survival for longer than 24 hrs. could occur even with a blood pressure as low as 50 mm. Hg, after a fall to 40 mm. Hg survival for more than 4 hrs. occurred in only one instance. 3. In the "ligated" animals death was apt to occur with a precipitous (in 15 min. or less) fall of blood pressure to zero from a level that was in 2 cases above 60, but which averaged 40 mm. Hg. It seems that 40 to 50 mm. Hg represents a critical pressure level for the rabbit in tourniquet shock.

*Fluid loss and hematocrit.* We performed ten experiments in which the tourniquet was applied to only one leg for two hours, then both legs were removed by careful dissection of the leg muscles away from the external surface of the ilium, the pubic arch and sacrum, followed by disarticulation of the hip joint. Thus the

masses of tissue removed on each side were roughly equivalent. The average gain in weight of the tourniqueted limb was  $1.07 \pm 0.12$  per cent of the body weight. Thus, when both legs were occluded, one could assume that there would have been a loss of fluid of about 2 per cent of the body weight, which might be the equivalent of at least 30 per cent of the whole blood or 60 per cent of the plasma. Undoubtedly this fluid loss, if taken from the blood, could in itself be ample cause of death.

However, the striking thing about the hematocrit observations was their constancy, the change in the control animals during the period of observation being  $2.9 \pm 0.8$ ; in the ligated animals  $0.1 \pm 1.2$ . Several observations were usually made during the course of each experiment, and the changes were nearly always negligible and apparently random. From this it appears that any fluid drawn from the blood into the occluded limbs was promptly made up from the tissues, the only net effect being a slight dehydration of the latter. We conclude therefore that the fluid loss was not the cause of shock in these experiments.

*Effect of dehydration and fasting.* Partly because of the evidence mentioned above of some tissue dehydration in the "ligated" animals we tested the effect of marked dehydration on the production of tourniquet shock. Seven animals who drank no water for 5 to 7 days and who refused food during the same period were subjected to 5 hrs.' tourniquet occlusion. Their survival time was  $2.5 \pm 0.1$  hrs., as compared with a survival time of 1.7 hrs. in the normally fed and hydrated animal. In view of the voluntary fasting by the dehydrated animals, six other rabbits were fasted for 5 to 7 days with water *ad lib*. The survival time in this group was  $1.9 \pm 0.3$  hrs. It appears therefore that neither dehydration nor fasting, resulting in an average loss of weight of over 20 per cent, influenced significantly the survival in tourniquet shock.

#### SUMMARY

1. Shock was produced in the rabbit by occlusion of the circulation in the hind legs.
2. After 2 hrs.' occlusion at room temperature, the survival time after tourniquet release was  $3.8 \pm 0.6$  hrs.; after 5 hrs.' occlusion it was  $1.7 \pm 0.4$  hr. Thus a technique with predictable results is offered for the study of shock.
3. Tourniquet shock in the rabbit is not due to loss of fluid in the ligated legs.
4. Chilling the tourniqueted legs more than tripled the average survival time in most experiments; in the remainder death was delayed as much as 24 hrs.
5. Dehydration and previous fasting did not affect the survival time.

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# THE EFFECT OF DICUMAROL UPON PLASMA FIBRINOGEN

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Roderick (1929) reported that the feeding of spoiled sweet clover hay to cattle resulted in a hemorrhagic tendency that was due to a decrease in the plasma prothrombin. In a later paper (Roderick, 1931) he noted that there was also a decrease in the plasma fibrinogen. The identification of the hemorrhagic factor in spoiled sweet clover hay as 3,3'-methylenebis (4-hydroxycoumarin) ("dicumarol") by Stahman, Huebner and Link (1941) has been followed by the repeated confirmation of the effect on plasma prothrombin. No confirmation of the effect on plasma fibrinogen, however, has been reported although Allen, Barker and Waugh (1942) have reported an increase in the sedimentation rate of the erythrocytes following the administration of dicumarol. This would suggest an increase in plasma fibrinogen, since the concentration of fibrinogen appears to be the most important single factor governing the sedimentation rate.

Dicumarol is now used clinically to produce a state of hypoprothrombinemia. However, the nature of its action is still quite unknown. The fact that dicumarol lowers the blood prothrombin by interfering with its production in the body, and the fact that the liver is necessary for prothrombin synthesis (Brinkhous, 1940) has aroused suspicion that dicumarol might act as a specific liver toxin. No evidence of such action has been observed with moderate therapeutic doses (Bingham, Meyer and Pohle, 1941; Wright and Prandoni, 1942). Such failure to observe an effect on liver might easily be due to the insensitivity of the tests, since it is well known that considerable damage can occur without any detectable histological changes. Ham and Curtis (1938) have emphasized the sensitivity of the plasma fibrinogen level as an index to liver function. The present study, therefore, was undertaken to determine if dicumarol had any effect on the plasma fibrinogen level.

**METHODS.** Dogs were used, and were selected on the basis of a reasonably constant plasma fibrinogen level. They were inspected daily for any detectable infection and special care was taken in making injections and removing blood samples to avoid possible causes of irritation or infection. The results were discarded if any infection or abscess developed during the course of an experiment. The animals were maintained on a diet of Purina Fox Chow. Blood samples were taken from the superficial veins. Plasma fibrinogen was determined by the method of Campbell and Hanna (1937) and the prothrombin time by the method of Quick (1938).

We are indebted to the Charles H. Frosst Company, Montreal, for an ample supply of dicumarol. This was dissolved in physiological saline immediately before use by adding a few drops of 5 N NaOH, and was injected intravenously.

**RESULTS.** Various doses of dicumarol (2.5–20 mgm./kgm.) were administered to a number of animals and their plasma fibrinogen determined daily. Pro-

thrombin times were also determined on most of the animals. To control variations in fibrinogen due to other factors, the fibrinogen was determined for 3 to 5 days before the experiment. In the experiments cited the fibrinogen returned to this "normal value" in 2 to 7 days after the injection. Controls showed that in animals thus standardized and free of infection the fibrinogen in this period of time did not vary more than  $\pm 0.02$  mgm. N/cc., so that greater variations could be ascribed to the dicumarol. However, longer periods of time showed a greater change in the fibrinogen level and the fibrinogen appeared to

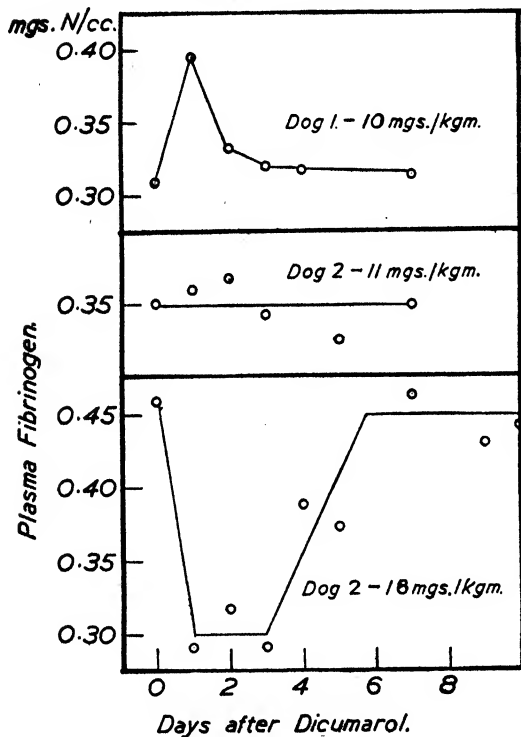


Fig. 1

Fig. 1. Plasma fibrinogen following the injection of dicumarol.

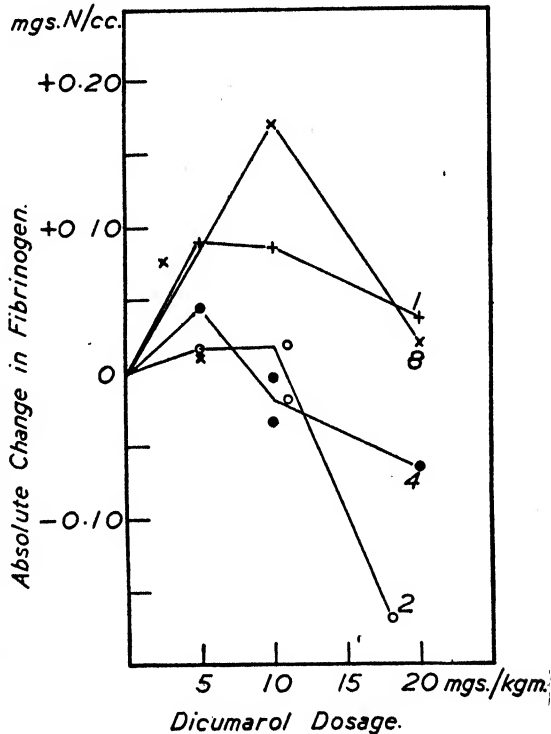


Fig. 2

Fig. 2. Effect of dosage of dicumarol on the change in plasma fibrinogen following dicumarol injection. + — + dog 1, O — O, dog 2, ● — ● dog 4, X — X, dog 8.

increase gradually with the animal's sojourn in the laboratory. Each experiment was followed by a rest period (usually about three weeks) so that there was considerable variation in the "normal value" for the same dog in different experiments.

Curves typical of those obtained are shown in figure 1. On administering 10 mgm./kgm. of dicumarol to dog 1, the fibrinogen rose from an initial value of 0.31 to 0.40 mgm. N/cc. 24 hours after the injection and then gradually returned to the initial value. In 11 experiments involving seven animals, a significant rise in fibrinogen occurred 24 hours after the injection. Eleven milligrams/kilogram was administered to dog 2 (fig. 1) but was followed by no significant change

in fibrinogen. When 18 mgm./kgm. was administered to this animal, the fibrinogen fell from 0.46 to 0.30 mgm. N/cc. where it remained for 2 days, gradually returning to normal.

The effect of graded doses of dicumarol is shown more fully in figure 2 by plotting the absolute change in fibrinogen occurring 24 to 48 hours after the injection. With dog 4, 5 mgm./kgm. caused a significant rise in fibrinogen; 10 mgm./kgm. in one experiment caused a significant decrease in fibrinogen, in a second experiment resulted in no significant change; 20 mgm./kgm., however, caused a marked decrease in fibrinogen. Hence in this dog, a 5 mgm. dose caused a rise, 10 mgm. little change and 20 mgm. a fall in fibrinogen. Similar results were obtained with other animals. However, the dosage at which a positive response was changed to a negative one depended on the animal. Thus with dogs 1 and 8 the fibrinogen response increased with dicumarol dosage up to 10 mgm./kgm. and it was the 20 mgm. dose which resulted in no change in fibrinogen. Dog 2, on the other hand, in which 18 mgm./kgm. caused the greatest fall in fibrinogen observed in the series, showed little change with 5 and 11 mgm. doses, suggesting that these doses were too large to cause an increase in fibrinogen in this animal. The experiments with all the other animals studied showed the same pattern of the change of fibrinogen response with dosage as those reported in figure 2. In view of the possibility that, in spite of the long period elapsing between doses, the response to a given dose might be affected by previous administration of dicumarol, the order in which the various doses of dicumarol were taken was varied with different animals. The same pattern of response was obtained in all cases, indicating that previous administration of dicumarol was not a factor.

All animals showed approximately the same change in prothrombin time with the dicumarol, the prothrombin time increasing with the dicumarol dosage. While there was some variation, it was not great enough to draw conclusions correlating the degree of lowering of prothrombin with the nature of the fibrinogen response. Of interest, however, was the finding that while the change in fibrinogen reached a peak 24 to 48 hours after the injection and returned to normal by the 2nd to 5th day, the maximum prothrombin time, as has been reported by others, was not reached until 3 to 5 days after the injection.

**DISCUSSION.** Fibrinogen has been found to be an extremely labile plasma protein constituent, and fluctuations in amount are caused by a number of factors. Foster and Whipple (1921) reported that with the most rigidly controlled conditions the fibrinogen of normal dogs shows considerable variation, but that under uniform conditions the blood fibrinogen of an individual adult normal dog will not show any great fluctuation. Severe liver necrosis produced low fibrinogen values, whereas slight liver injury served as a stimulus for the production of fibrinogen. Schultz, Nicholas and Schaeffer (1925) found an immediate rise in fibrinogen after mild liver injury. These findings have been confirmed for many conditions, both experimentally and clinically (cf. Ham and Curtis).

The results obtained with dicumarol show the same pattern and suggest that the drug is toxic to the liver. Small doses may cause mild liver injury and stimulate the production of fibrinogen, and larger doses may cause more severe injury and depress the formation of fibrinogen. The results obtained are in agreement

with data from the original experiments of Roderick, since not only does he report a fall in fibrinogen but an examination of his data suggests that in several cases this fall in fibrinogen was preceded by a slight rise at the beginning of the feeding experiments.

The change in fibrinogen during the first 24 to 48 hours following the injection of dicumarol indicates that the action of the latter upon the liver is immediate. This has been previously suggested for the effect of dicumarol on prothrombin, the lag in the latter response being ascribed to the time required for the circulating prothrombin to be used up. If the latter view is correct then the difference in the times at which plasma fibrinogen and prothrombin decrease after dicumarol is due to differences in the rate of utilisation by the body rather than to differences in the action of dicumarol in the hepatic cells involved in the production of these substances.

#### SUMMARY

Plasma fibrinogen was determined in dogs injected with varying amounts of dicumarol. The administration of dicumarol has an effect on plasma fibrinogen as well as on plasma prothrombin. Large doses tend to lower the plasma fibrinogen concentration; smaller doses raise it. These results are similar to those obtained with liver toxins.

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# THE PHYSIOLOGICAL BILATERALITY OF THE PORTAL CIRCULATION

## STREAMLINE FLOW OF BLOOD INTO THE LIVER AS SHOWN BY RADIOACTIVE PHOSPHORUS<sup>1</sup>

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It was suggested by Serege (1) in 1902 that the blood entering the liver from the portal circulation did so in more or less discrete channels of streamline flow. Thus the blood from the spleen was largely diverted to the left side of the liver while that from the mesenteric veins tended to move to the right side.

By the introduction of small amounts of radioactively tagged phosphoric acid into the various contributory branches of the portal vein it is possible to demonstrate that such a streamline flow takes place without considerable mixing of the blood from the various organs in the path of the fluid. Using this method we have carried out several experiments which demonstrate this phenomenon. The results are presented in figure 1.

**METHODS.** Three healthy adult mongrel stock dogs were used in the experiments. In experiment 1 the animal was anesthetised with nembutal (28 mgm. per kgm. body weight). A mid-line incision was made extending from the xyphoid process to the umbilicus and the splenic vein was exposed. Loose ligatures were placed about the portal vein, hepatic artery, and inferior vena cava. About 0.5 mgm. of tagged phosphorus in the form of  $H_3PO_4$  having about 0.5 millicurie of radioactivity and contained in about 0.5 ml. of solution was injected in the splenic vein as near the spleen as possible. After approximately three seconds' interval the ligatures were pulled and tied. The liver was removed intact and immediately frozen in an ether-dry mixture. Transverse sections were then cut across the liver and after being weighed were wet ashed in sulphuric and perchloric acids (2). After neutralisation with concentrated sodium hydroxide the samples were made to a convenient volume in volumetric flasks and suitable aliquots were examined for labelled phosphorus. Radioactivity measurements were carried out on a counting rate meter<sup>2</sup> such as described by Gingrich and Evans (3, 4). The aliquots taken had counts of the order of 1,000 to 10,000 per minute with a background count of about 55 per minute.

In experiment 2 the tagged phosphoric acid was injected into the jugular vein and about five minutes were allowed to elapse before tying off the hepatic vessels. This animal served as a control on the other two experiments, since fairly complete mixing of the labelled phosphorus in the circulation was allowed and no difference in concentration of the tagged material should have existed in the blood from the contributing vessels in the portal circulation.

<sup>1</sup> This work was made possible through a grant from the Nutrition Foundation.

<sup>2</sup> Measurement equipment was provided by the Bristol-Meyers Company.



In experiment 3 the tagged phosphoric acid was injected in a branch of the mesenteric vein, about three seconds being allowed to elapse before tying off the loosely ligated hepatic vessels.

**EXPERIMENTAL OBSERVATIONS.** The results of these three experiments can best be seen by examination of figure 1 in which the percentage of the total tagged phosphoric acid found, per gram of liver appearing in each slice, is plotted against the per cent of liver mass traversed from the right to the left side of this organ. Due to the irregularity in the shape of this organ and the variability in its thickness the results can be looked on as being only semi-quantitative. However, these variabilities are minimised in opposite paired sections of the liver. The differences in the findings following injection in the splenic and mesenteric veins are so great when compared with the control experiment and when compared with

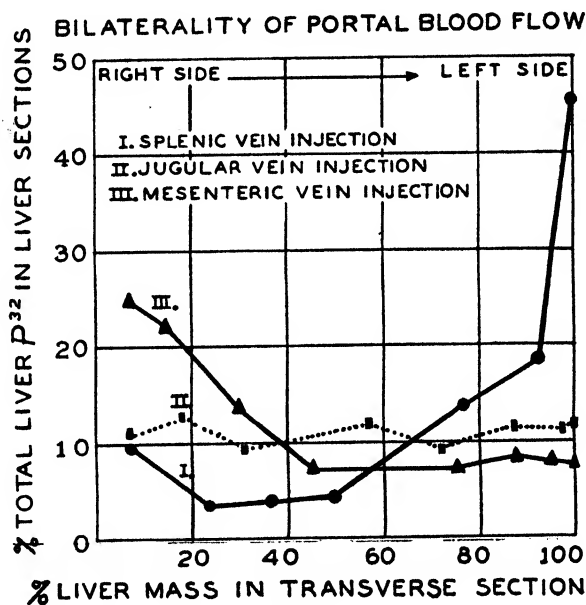


Fig. 1

one another, that one can hardly escape the conclusion that there is definite evidence of the bilaterality of flow of blood in this part of the circulation. In the case of the splenic vein injection there was about 4.5 times the amount of tagged material in the *left* extreme section per gram of hepatic tissue as on the corresponding right side. In contrast, in the dog injected in the branch of the mesenteric vein there was about 3 times as much labelled phosphoric acid in the *right* section as contrasted with the left section. The control animal showed *equal* distribution of the tagged phosphorus in all sections taken per weight of liver tissue within the limits of error of the methods employed, there being no apparent differences on either side.

**DISCUSSION.** The greatest interest which is associated with these findings

would likely be in respect to the implications concerning metastatic involvement of hepatic tissue from primary tumors in the mesenteric areas, with an expected localisation of the secondary process in the right side of the liver. Occasionally at autopsy one observes metastases in the right side of the liver due to intestinal malignancy.

It seems doubtful that the existence of a streamline flow from the vessels contributing to the portal supply should in any manner alter noticeably the composition of the liver from a chemical point of view since in order to do so one would be forced to hypothesize that the removal of such materials from the blood passing through this organ is much more rapid than probably obtains. However, in the case of particulate matter such as tumor fragments, the situation is extremely efficient and one trip through the organ would likely suffice to remove most of the suspended material.

#### SUMMARY

By the use of phosphoric acid tagged with the radioactive isotope it is shown that when the material is injected in the splenic vein there is a decidedly greater concentration of the tagged phosphorus in the left side of the liver than in the right side within about three seconds of the time of injection. In contrast, when the same material is injected in a branch of the mesenteric vein there is a markedly greater accumulation of the labelled material on the right side of the liver. When it is injected into the jugular vein and sufficient time allowed for complete mixing with the circulating blood there are no demonstrable differences in concentration of the tagged material in various transverse sections taken from the liver.

There is evidently a physiological bilaterality of flow of blood in the portal vein which is streamline in nature and by which splenic blood is largely sent to the left side and mesenteric blood to the right side of the liver.

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## RENAL FUNCTION IN DOGS UNDER ETHER OR CYCLOPROPANE ANESTHESIA

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Renal function in unoperated dogs anesthetized with sodium pentobarbital was investigated by means of clearance methods by Corcoran and Page (1). In most of their anesthetics renal blood flow and glomerular filtration were essentially normal. In a series of patients studied by Collier, Rees, Campbell, Job and Moyer (2), the combination of anesthesia by ether or cyclopropane with surgical operation sometimes had no effect on renal function and sometimes depressed renal blood flow and glomerular filtration. They attributed the changes in renal function so often seen in anesthesia to alterations outside the kidney.

The present experiments were undertaken to observe renal function in dogs under ether or cyclopropane anesthesia in the absence of the complications introduced by surgical operation. Clearances of creatinine ( $C_{Cr}$ ) and sodium p-aminohippurate ( $C_{PAH}$ ) were used to measure glomerular filtration and renal plasma flow respectively; in most cases the maximum tubular reabsorption of glucose from the glomerular filtrate ( $Tm_G$ ) was also measured.

In series I clearances were first determined on unanesthetized dogs; anesthesia was then induced and additional clearances were determined under anesthesia. The results duplicated those of the previous investigators (1, 2), for in some anesthetics there was no change from the control values, while in others the clearances were markedly depressed.

In series II the influence of the depth of anesthesia was examined systematically. The experiments were conducted in three successive phases: first under light anesthesia, second under deep anesthesia, and third under light anesthesia again. Reversible depression of function was usually seen in the second phase.

**METHODS.** Ten female dogs ranging from 8 to 16 kgm. in weight were used. The position of the dogs during the experiments was supine on a padded dog board and a minimum of restraint was applied. Well trained dogs were not used for the first experiments because of the hazard of anesthesia. For this reason good relaxation was not always obtained in the control periods before anesthesia. Moreover, some trained dogs did not relax and appeared apprehensive when returned to the board after one or more anesthetics.

At the beginning of the experiment the dogs were given about 50 cc. per kgm. of water by mouth. Creatinine, PAH and a diuretic (mannitol or glucose) were infused at constant rate into an ear vein; a priming dose of each compound was given at the beginning to supplement the infusion. In series I various infusions were employed, but in series II only one. Here the priming infusion

contained 1 gram of creatinine, 10 grams of glucose and 0.75 cc. of a 20 per cent solution of PAH made up to a volume of 20 cc. with water. The sustaining infusion contained 2 grams of creatinine, 60 grams of glucose and 4 cc. of a 20 per cent solution of PAH made up to a volume of 300 cc. with 0.85 per cent NaCl solution. The rate of infusion in series II was about 1.75 cc. per minute.

Urine was collected at measured intervals of about 10 minutes by means of an indwelling catheter. The bladder was washed out at each collection with 10 cc. of water. Blood was drawn from the jugular vein on the side away from the infusion or from a femoral artery if glucose was being determined. At least two samples of blood were drawn for every three urine collection periods. Blood pressure was read from a mercury manometer following puncture of a femoral artery. Rectal temperature, respiratory rate and heart rate were noted at intervals.

*Anesthesia.* Ether anesthesia was induced by the open drop method, and when it was sufficiently deep, an endotracheal tube was inserted and connected to a rubber bag for to and fro breathing by means of a soda lime canister to absorb carbon dioxide. Oxygen was supplied continuously and ether intermittently from a metric gas machine.<sup>1</sup> A depth of anesthesia was sought that would prevent swallowing and limb movements but maintain the eye reflexes and costal respiratory movements. Unintentional deviations from this level were usually on the deep side.

Cyclopropane anesthesia was induced by means of a cone supplied with a 50-50 mixture of cyclopropane and oxygen. At the appropriate depth the endotracheal tube was inserted and connected with the rebreathing equipment used for ether. Oxygen was supplied continuously and cyclopropane intermittently. The depth of anesthesia was regulated to maintain the respiratory movements at a low rate. It was difficult to maintain a constant level of anesthesia with cyclopropane and with some dogs there were intermittent periods of artificial respiration given by squeezing the bag.

In series I the plan was to maintain the level of anesthesia at the first plane of the surgical stage. However, sufficient care was not taken to prevent considerable fluctuation toward the third plane both during the time between induction and the beginning of the clearance periods, and during these periods. In series II, the depth throughout the anesthesia was more carefully controlled, and advantage was taken of the experience gained in series I. The time between induction and the beginning of the collection periods was kept to a minimum. During this time and in phases 1 and 3, care was taken to maintain the activity of the eyelid and corneal reflexes. In phase 2, the level of anesthesia was deepened to the point of elimination of costal breathing with ether, and cessation of both costal and diaphragmatic breathing with cyclopropane.

*Chemistry.* The blood samples were collected with a minimal amount of saturated potassium oxalate and centrifuged for 10 minutes. The plasma protein

<sup>1</sup> This machine was very generously made available to us from the Department of Anesthesia by Dr. E. A. Rovenstine. Doctor Rovenstine very kindly demonstrated the intubation and the use of the equipment with each anesthetic agent.

was precipitated with  $\text{CdSO}_4$  (3). These  $\text{CdSO}_4$  filtrates and the diluted urine samples were placed in the refrigerator and analysed in duplicate the following day.

Creatinine was determined according to the alkaline picrate method (4); the colorimeter readings were made exactly 10 minutes after addition of the picrate, with a 520 filter. Mannitol clearances were determined simultaneously with creatinine in the first four experiments; the mannitol was determined by the method of Smith, Finkelstein and Smith (5). The ratio of the mannitol and creatinine clearances was  $1.04 \pm 0.029$  for 9 control periods and  $0.99 \pm 0.026$  for 17 periods under ether. Since the ratio did not change significantly under ether, it was considered that the creatinine clearance gave a satisfactory measure of glomerular filtration under these conditions.

Determination of PAH was carried out by a modification of the method of Bratton and Marshal (6) as described by Smith, Finkelstein and Aliminosa (7). The assumption implicit in the use of  $C_{\text{PAH}}$  as an index of renal blood flow is that any decline in  $C_{\text{PAH}}$  in anesthesia represents a change in blood flow and not in the degree of extraction of PAH. The degree of extraction of PAH from the blood by the kidney as estimated from clearance methods (7) has been confirmed by analysis of renal vein blood from explanted kidneys of dogs anesthetized with sodium pentobarbital by Phillips et al. (8). In their experiments the extraction ratio averaged 0.85. In normal human subjects whose renal vein blood has been collected, somewhat higher average extraction ratios have been obtained: 0.92 (9) and 0.88 (10).

Glucose was determined by the method of Folin as modified by Shannon, Farber and Troast (11). When the results for  $\text{Tm}_G$  were calculated on the basis of true glucose by yeast fermentation (12) the average deviation from the values obtained without yeasting was  $2 \pm 1$  per cent for 52 periods in 9 experiments. For this reason the yeast treatment was abandoned.

**RESULTS. Series I.** The data for this series are summarized in table 1. The clearance data have been corrected for surface area (13) and plotted in figure 1 against a statistical background provided by a series of 203 unpublished control observations on 52 normal female dogs collected over a period of years in this laboratory and collated by one of us (C. R. H.). The hexagon in the figure includes all points whose deviation from the means for filtration fraction,  $C_{\text{Cr}}$  and  $C_{\text{PAH}}$  is less than twice the standard deviation of the mean. This area will be regarded as the normal range of variation.

Of the 17 control points in series I, 5 include at least one clearance (either  $C_{\text{Cr}}$  or  $C_{\text{PAH}}$ ) outside the normal range, 6 have elevated filtration fractions only, and 6 fall within the normal range in all respects. Of the 6 points having an elevated filtration fraction 4 (expts. 7, 9, 11, 13) come from dog Gt who did not like to lie on her back.

Of the 18 points representing anesthetics, 8 fell within the normal range, one had a slightly elevated filtration fraction, and 9 exhibited a depression in either  $C_{\text{Cr}}$ ,  $C_{\text{PAH}}$  or both. The general trend of the filtration fractions was somewhat higher than the mean of the normal range. There was no pronounced change in

TABLE 1  
*Renal clearances before and during anesthesia with ether or cyclopropane*  
 Series I

EXP. NO.	DOG		DATE	AGENT	TIME FROM INDUCTION TO 1ST PERIOD	NUMBER OF PERIODS	BLOOD PRESSURE	URINE FLOW	C <sub>Cr</sub> *	C <sub>PAH</sub> *	FILTRATION FRACTION* C <sub>Cr</sub> /C <sub>PAH</sub>	
	Weight	Surface area										
		kg.	sq. m.				mm. Hg	cc./min.	cc./min.	cc./min.		
1	C	16.5	0.86	8/18	None	3		2.47	61	169	0.361	
2				Ether	111	3	1.30	32—	132—	0.234		
3				8/26	None	3	101	1.00	79	247	0.319	
4				Ether	165	1	62, 86	0.66	12—	108—	0.112—	
5				9/15	Ether	201	6	74	0.84	46	203	0.227
6				9/23	None	3	108	2.67	60	175	0.345	
7	Gt	11.2	0.69	9/8	None	5	105	1.92	52	128	0.414+	
8				Ether	82	5	90	2.44	54	163	0.333	
9				1/10	None	3	138	2.86	53	114	0.460+	
10		10.9	0.59		Ether	118	3	91	5.88	43	107	0.400+
11				1/17	None	2	130	1.06	46	105	0.440+	
12				Ether	140	3	70	1.91	19—	51—	0.380	
13		10.9	0.59	2/17	None	3	135-115	4.29	55	131	0.417+	
14				Ether	75	3	108	5.22	60	170	0.353	
15				2/29	Ether	109	3	91	5.24	36	101	0.373
16	B	9.3	0.57	1/27	None	4	110	3.46	77+	171	0.455+	
17				Ether	105	1	101	1.25	23—	46—	0.480+	
18	Gl	12.0	0.71	1/31	None	3	110-140	8.86	50	105—	0.477+	
19				Ether	101	3	126	3.00	22—	66—	0.337	
20	K	12.8	0.69	2/7	None	6	120	9.72	39	112	0.350	
21				Ether	81	3	120	12.00	33	78—	0.423+	
22	D	8.5	0.49	2/15	None	3	120	1.97	24	77—	0.353	
23				Ether	71	3	120	1.07	31	83	0.370	
24				3/6	None	3	130	2.88	23	58—	0.429+	
25				Cyclo	160	3	75-130	0.84	23	54—	0.400+	
26	H	10.9	0.55	2/21	None	6	120	4.61	65	247+	0.263	
27				Ether	67	3	96	0.37	8—	96	0.085—	
28				3/2	None	3	100-115	2.98	58	146	0.398+	
29				Cyclo	138	3	102	2.17	31	77—	0.414+	
30	T	8.1	0.51	2/24	None	3	100-140	4.30	60	194	0.307	
31				Ether	60	3	100	1.83	59	197	0.298	
32				3/9	None	3	110-150	4.09	51	141	0.359	
33				Cyclo	89	3	109	3.74	40	115	0.350	
34	N	11.9	0.61	3/13	None	3	100	4.61	52	117	0.421+	
35				Cyclo	145	3	120	4.30	53	148	0.355	

\* The addition of + or — indicates the figure is outside the normal range as explained in figure 1.

filtration fraction as a result of anesthesia, although 14 out of 18 were lower during anesthesia than during the control periods.

Data for  $Tm_G$  are given in table 2. In 5 out of 7 experiments,  $Tm_G$  was lower under anesthesia than in the control periods and in 3 the difference was greater

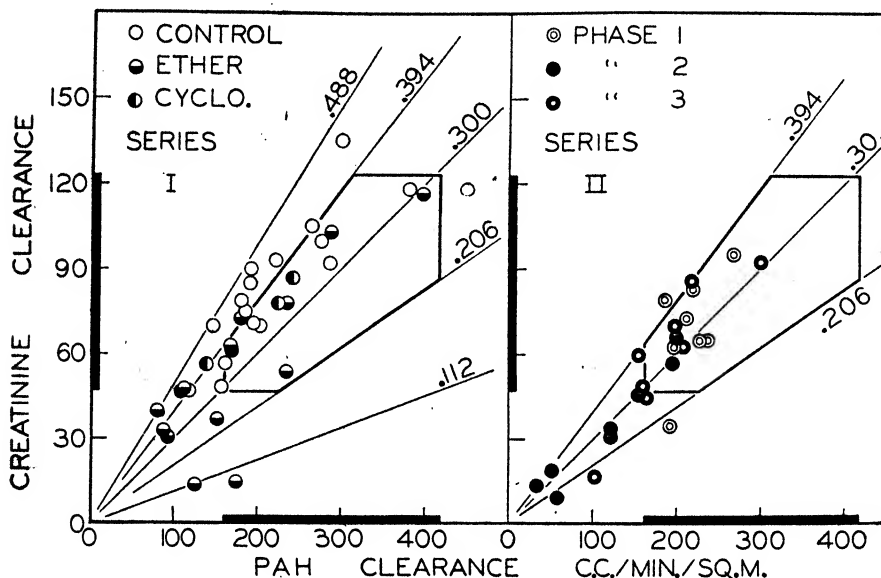


Fig. 1. Clearances of creatinine and p-aminohippurate in units of cubic centimeters of plasma per minute per square meter of body surface area.

*Statistical background.* This is derived from a control series of 203 observations including two or more clearance periods made previously by other workers in this laboratory on 52 normal female dogs. The means and standard deviations were  $85 \pm 19$  for  $C_{Cr}$ ,  $290 \pm 64$  for  $C_{PAH}$  and  $0.300 \pm 0.047$  for the filtration fraction. The solid columns on the ordinate and abscissa represent  $\pm$  twice the standard deviation from the means. The diagonals represent the mean filtration fraction and other filtration fractions at intervals of twice the standard deviation. The hexagon made by the heavy lines defines the limits of twice the standard deviation from the three means. It includes 90 per cent of the observations of the control series and is referred to in the text as the normal range.

*Series I.* The data for the dogs of table 1 unanesthetized (open circles) and anesthetized with ether or cyclopropane, corrected for surface area and plotted against the statistical background of the control series.

*Series II.* The data of table 2 corrected for surface area and plotted against the statistical background of the control series. The three phases refer to successive conditions of light, deep and light anesthesia, as described in the text. Four experiments with ether and four with cyclopropane are included.

than 10 per cent. In these 3 instances the load was maintained well above  $Tm$  levels but the  $C_{Cr}$  fell.

*Series II.* The detailed data are collected in table 3, and the clearance data corrected for surface area are shown in figure 1. In phases 1 and 3, 6 out of 8 points fell within the normal range; in phase 2 under deep anesthesia both clearances were reduced so that out of 8 points only 2 remained within the normal

range. In one of these (expt. 39)  $C_{Cr}$  was depressed 21 per cent and  $C_{PAH}$  only 9 per cent, although the urine flow fell by 52 per cent and the blood pressure fell to 70 mm. Hg. In the other (expt. 37) the clearances were still within the normal range although  $C_{Cr}$  and  $C_{PAH}$  fell by 40 and 27 per cent respectively and the urine flow decreased by 59 per cent.

With the exception of experiment 39, just cited, and experiment 37, in which the blood pressure was not observed in phase 2, the decrease in  $C_{PAH}$  in phase 2 occurred without significant change in the systemic arterial pressure.

TABLE 2  
*Effect of anesthesia on  $Tm_G$*   
Series I

DOG	DATE	AGENT	NUMBER OF PERIODS	$C_{Cr}$	$PG^*$	LOAD $PG_{Cr}$	$Tm_G$	$Tm_G$ ANESTHETIZED
								$Tm_G$ CONTROL
Gt	1/17	None	1	46	3.91	180	127	1.11
		Ether	1	29	9.60	278	141	
Gt	2/17	None	3	55	4.42	243	136	0.91
		Ether	3	60	5.77	346	124	
B	1/27	None	4	77	3.79	292	165	0.88
		Ether	1	23	8.90	205	145	
Gl	1/31	None	3	50	10.50	525	138	1.01
		Ether	1	28	14.60	410	140	
H	3/2	None	3	58	5.54	321	124	0.82
		Cyclo.	3	31	7.32	227	102	
T	3/9	None	3	51	5.67	289	117	0.83
		Cyclo.	3	40	6.49	259	98	
N	3/13	None	3	52	4.02	209	117	0.92
		Cyclo.	3	53	5.64	299	108	

\* In each of the above experiments there was no significant change in either rate or composition of infusion from the control periods to the periods under anesthesia. Accordingly the rise in blood sugar is regarded as an effect of the anesthetic agent. The commonly observed hyperglycemia of ether is explained only in part by sympathetic excitation (14).

The data for series II are summarized in table 4. The values for phases 2 and 3 were converted to a percentage of the value for phase 1 before being averaged. In general the increased depth of anesthesia in phase 2 was accompanied by a decrease of about one-half in urine flow,  $C_{Cr}$ ,  $C_{PAH}$  and  $Tm_G$ , and no change in filtration fraction. In many cases recovery in phase 3 was incomplete, but for the series the difference between phase 1 and phase 3 was not statistically significant.

In series II decreases in  $Tm_G$  were observed in spite of the fact that in every



**TABLE 3**  
*Effect of deep anesthesia on kidney function*  
**Series II**

Phase 1, stage III plane 1; phase 2, stage III plane 3; phase 3, stage III plane 1.

EXPT. NO.....		36	37	38	39	40	41	42	43
Dog.....		H		Gt			M		
Weight, kgm.....		10.5	10.1	10.9	10.4	10.1	11.3	11.8	11.2
Surface area, sq. m.....		0.63	0.61	0.62	0.62	0.62	0.63	0.63	0.63
Date.....		3/31	4/20	4/6	4/27	5/11	4/14	5/5	5/18
Agent.....		Ether	Cyclo	Ether	Cyclo	Cyclo	Ether	Cyclo	Ether
Hydration, cc.....		0	500	500	500	500	300	550	550
Infusion, cc./min.....		1.75	1.75	1.94	1.80	1.59	1.72	1.75	1.80
Time of induction.....		9:35	1:36	9:55	1:50	1:30	1:40	9:48	1:43
Min. after induc- tion	Phase 1	62	64	71	64	40	57	47	57
	Phase 2	80	96	91	84	70	78	67	78
	Phase 3	118	115	122	104	111	98	87	97
	End of phase 3	163	165	176	155	146	160	138	152
Number of collection periods	1	2	3	2	2	3	2	2	2
	2	3	1	3	2	4	2	2	2
	3	4	5	5	5	3	4	5	5
Urine flow cc./min.	1	1.10	2.82	3.49	5.20	2.57	4.37	2.80	2.26
	2	0.41	1.16	1.73	2.50	0.93	2.55	0.60	0.82
	3	1.04	2.42	3.40	3.37	3.12	5.18	5.30	2.38
C <sub>Cr</sub> cc./min.	1	22	58	40	52	45	41	50	40
	2	6	35	21	41	20	29	9	12
	3	11	56	28	53	37	40	45	31
CPAH cc./min.	1	121	163	144	136	131	143	117	124
	2	37	119	75	124	75	97	21	39
	3	65	183	101	134	96	132	124	101
Filtration fraction	1	0.185	0.361	0.275	0.386	0.351	0.286	0.428	0.326
	2	0.172	0.294	0.274	0.337	0.265	0.297	0.398	0.282
	3	0.172	0.311	0.284	0.393	0.382	0.300	0.368	0.312
Load mgm./min.	1	173	211	322	289	237	293	254	276
	2	37	173	194	243	120	232	52	86
	3	123	296	314	321	273	339	292	223
Tmg mgm./min.	1	102	117	125	123	121	97	126	108
	2	33	100	79	119	66	89	26	34
	3	52	140	98	133	96	87	107	83
Blood pressure mm. Hg	1	96	110	101	114	90	95	105	94
	2	99		97	70	92	100	102	114
	3	96	99	100	99	123	105	125	102
Rectal temperature °C.	1	36.7	38.0	37.8	37.4	37.0	38.4	38.6	39.2
	2	36.9		37.1	36.7	36.3	38.3	38.3	39.3
	3	37.2	37.7	36.0	36.7	35.4	37.8	38.3	39.3

instance except experiment 36, phase 2, the simultaneous load/ $T_G$  ratio was above 1.25, the ratio ordinarily considered adequate for saturation (15). In experiment 38, phase 2, the load/ $T_G$  ratio was 2.46 and the load was 1.55 times the  $T_{MG}$  in phase 1, when  $T_{MG}$  and  $C_{Cr}$  were reduced by 37 and 46 per cent respectively from phase 1. In phase 3 there were 4 cases (expts. 36, 38, 40, 43) in which  $T_{MG}$  failed to recover to 80 per cent of phase 1. Here the load was adequate for saturation, but  $C_{Cr}$  was depressed as much or more than  $T_{MG}$ . In these four experiments  $C_{PAH}$  did not recover completely either; the ratio of phase 3 to phase 1 was 0.54, 0.70, 0.73 and 0.81 respectively.

Another activity for which data are available is the degree of reabsorption of water from the glomerular filtrate. The urine volume represents the fraction of the glomerular filtrate that fails to be reabsorbed. The ratio urine volume/ $C_{Cr}$  thus provides an indirect measure of water reabsorption. In series II this ratio did not change significantly from phase 1 to phase 2 (table 4). In recovery from phase 2 to phase 3, the ratio increased in each case and the mean increase

TABLE 4  
Statistical summary  
Series II

Means and standard errors of the means for phase 2 and phase 3 when phase 1 is set equal to 100. In the last line, phase 2 is set equal to 100.  $N = 8$ .

PHASE.....	1	2	3
Urine flow.....	100	41 $\pm$ 3.6	110 $\pm$ 13.1
$C_{Cr}$ .....	100	48 $\pm$ 7.7	83 $\pm$ 6.0
$C_{PAH}$ .....	100	53 $\pm$ 8.9	86 $\pm$ 7.0
Filtration fraction.....	100	91 $\pm$ 3.2	98 $\pm$ 3.2
$T_{MG}$ .....	100	60 $\pm$ 10.5	86 $\pm$ 7.4
Urine flow/ $C_{Cr}$ .....	{ 100	95 $\pm$ 9.5 100	137 $\pm$ 17.0 142 $\pm$ 9.4

was 42 per cent. Such a relative increase in urine flow in recovery might be expected in order to compensate for the absolute reduction in flow during the phase of deep anesthesia (16).

DISCUSSION. From the present experiments and the observations of others it is evident that renal function is not necessarily impaired under light anesthesia. In series I  $C_{Cr}$  or  $C_{PAH}$  was depressed below the normal range in 7 anesthetics with ether and 2 with cyclopropane. In these experiments the protocols provide some additional information about the condition of the animal during the clearance periods but not during the preliminary time under anesthesia, namely:

1. Anoxia, as indicated by dark arterial blood or a blue tongue in experiments 4, 17 and 27
2. Deep anesthesia, continuous or intermittent, experiments 12 and 25
3. Depressed respiration, experiments 19 and 21 (here the blood sugar was unusually high due to rapid infusion, the maximum levels being 1895 and 2650 mgm. per 100 cc. respectively)
4. No indication, experiments 2 and 29.

Notes on the 9 anesthetics in which the clearances were within the normal range show:

1. Anoxia (dark blood or blue tongue) in experiments 33 and 35
2. Three inductions before intubation was accomplished, experiment 5
3. Low rectal temperatures, 35.8°C. in experiment 10, and 32.7°C. in experiment 15
4. No indication in experiments 8, 14, 23 and 31.

It is possible that errors in judging the depth of anesthesia were made during the clearance periods. It is also possible that the anesthesia was maintained at the desired level during the clearance periods but had been permitted to deepen during the interval between induction and the beginning of the clearance periods. This raises the question of the reversibility of the changes in renal function in deep anesthesia such as were seen in series II. The recovery of function in phase 3 was rapid and complete in 4 cases out of 8, but in experiments 36, 38, 40 and 43, recovery was only 80 per cent or less of phase 1, and the depression in function persisted throughout phase 3 or for 30 to 50 minutes. Repeated changes in depth of anesthesia place an additional strain on the circulation. Bennett, Bassett and Beecher (17) found that repeated deepening of anesthesia hastened the development and increased the severity of shock induced in dogs by hemorrhage.

One striking feature of both series was the stability of the filtration fraction at times when the clearances fluctuated over a wide range. In series I an elevated filtration fraction was brought within the normal range in experiments 8, 12, 14, 19 and 35, but not in 10, 17, 25 and 29. A low filtration fraction was seen in experiments 4 and 27 in association with renal shutdown. In series II phase 2, the filtration fraction fell in the average by only 9 per cent while the clearances fell by about 50 per cent.

The deeper levels of anesthesia were characterized by decreased renal clearances and no important changes in blood pressure or filtration fraction. Deep anesthesia thus resembles traumatic shock (18) which is characterized by decreased blood pressure and renal blood flow and by no change or a fall in filtration fraction, rather than sympathetic stimulation (19) which is characterized by a rise in blood pressure and filtration fraction and a fall in renal blood flow, or a sudden change in posture from supine to nearly upright brought about by a tilt table (20), which is characterized by a rise in blood pressure and a fall in both glomerular filtration and renal blood flow.

Under these circumstances it is likely that the decreased clearances in deep anesthesia are brought about by constriction of the afferent arterioles. Furthermore, the constriction is assumed to be neurogenic. The question may be raised whether the vasoconstriction is generalized or whether some nephrons are rendered more ischemic than others. The data for  $Tm_G$  bear on this point. As pointed out above, there were a number of experiments in which depressed  $Tm_G$  under anesthesia was associated with a fall in  $C_{Cr}$  (series I, expts. 17, 29 and 33, and series II, phase 3, expts. 36, 38, 40 and 43). This suggests that the filtration rate was diminished considerably in a number of nephrons and not in others. The constriction in the vessels supplying these nephrons must have been

reversible and not productive of permanent damage, for repeated anesthetics failed to alter  $Tm_G$  in dogs Gt, H and M. A generalized decrease in  $C_{Cr}$  of the magnitude encountered here would not be enough in itself to affect  $Tm_G$  appreciably. Shannon and Fisher (21) showed that  $Tm_G$  fell only 13 per cent in one experiment after decerebration under ether when the blood pressure was lowered to 50 to 66 mm. Hg by means of a clamp on the aorta and  $C_{Cr}$  fell 50 per cent.

If the decreased urine flow and clearances found in deep anesthesia are due to neurogenic vasoconstriction, an explanation is provided for the commonly observed diuresis or hyperemia following renal denervation in anesthetized animals. In order to permit denervation in the 30 experiments beginning with those of Claude Bernard in 1859 that Smith (20) has reviewed, the anesthesia must have been fairly deep, deep enough to depress renal blood flow and urine flow. Under these conditions denervation might be expected to release the vasoconstriction and so permit the observed increase in urine flow or renal blood flow. If this explanation is correct it brings the acute experiments on renal denervation under anesthesia into harmony with those (also reviewed by Smith, 20) on renal function in unanesthetized subjects after renal denervation and in subjects denervated by spinal anesthesia. In these last two groups of experiments, denervation was accompanied by no significant change in renal blood flow.

#### SUMMARY

The present results for ether and cyclopropane in dogs agree with previous observations on ether and cyclopropane in man, and sodium pentobarbital anesthesia in dogs in that renal blood flow, glomerular filtration, and tubular activity as measured by clearance methods, are not changed significantly in light anesthesia.

In dogs under deep anesthesia with ether or cyclopropane, depression of renal function occurs. In experiments uncomplicated by surgical operation, renal clearances were determined in three consecutive phases, 1 under light anesthesia (stage III, plane 1), 2 under deep anesthesia (stage III, plane 3) at a level often used in surgical operations, and 3 under light anesthesia again. In four experiments with ether and four with cyclopropane, there were decreases in urine flow, glomerular filtration, renal blood flow, and tubular reabsorption of glucose in phase 2 amounting to about one-half the rates observed in phase 1; in phase 3, the recovery was substantially complete. Femoral mean arterial blood pressures measured at intervals did not change significantly from one phase to another.

The reduction in renal blood flow and filtration rate in deep anesthesia are attributed to neurogenic constriction of the afferent arterioles of the kidney. This hypothesis would explain in part the phenomenon of renal hyperemia following denervation, recorded in many older acute experiments; it would help to account for the fact that under better controlled conditions such denervation hyperemia is not demonstrated.

We are indebted to Dr. H. W. Smith for suggesting this problem, to Dr. E. A. Rovenstine for his interest and material help, and to Mr. R. B. Golbey for able assistance.

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# MUSCLE TONUS AS A FACTOR IN HEMORRHAGE AND SHOCK IN DOGS UNDER BARBITAL ANESTHESIA<sup>1</sup>

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The hypothesis that a decreased muscle tonus is the initiating factor in traumatic or hemorrhagic shock is entertained and defended by some workers (1, 2). The evidence supporting such a hypothesis is based chiefly on changes in intramuscular pressure during various manipulations which result in shock, or a shock-like state (2, 3). These studies assume that intramuscular pressure is a measure of muscular tonus. The validity of such an assumption has not been proven; on the contrary, similar measurements of intramyocardial pressures have proven unsatisfactory (4). However, Gesell, Blair and Trotter (5) reported a palpable softening of the skeletal muscle in unanesthetized dogs subjected to hemorrhage which they interpreted as a loss of muscle tonus. In a later paper Gesell and Moyle (6) failed to find consistent changes in resting muscle length during hemorrhage.

It appeared to us that dogs anesthetized with morphine and sodium barbital exhibited little or no tonus of the skeletal muscles even before instituting shock producing procedures. If this were true, then the loss of muscle tonus could not be an initiating factor, or even a contributing factor, in the production of shock in the anesthetized dog. The few experiments reported here confirm our observation.

**METHODS.** The most convincing evidence of changes of muscle tonus is derived from measurements of tension changes in the resting muscle. Such a method has been employed in this study. Dogs were anesthetized with morphine (3 mgm./kgm.) and sodium barbital (ca 200 mgm./kgm.), according to the method used in this laboratory (7). Mean blood pressures were recorded from one femoral artery and blood drawn from the other. A hind limb of the dog was rigidly immobilized by placing drill rods through the upper and lower femur and mid-portion of the tibia and clamping the heads of the rods to an arrangement of heavy bars so that the gastrocnemius muscle and its tendon were in a horizontal position. The tendon was attached to an optically recording isometric muscle lever of good linear characteristic (8). Various precautions were taken to insure that artifacts did not occur due to the slipping of the lever or its connections.

**RESULTS AND DISCUSSION.** As a control measure the natural changes in muscle tension in anesthetized dogs were recorded over a period of hours in three experiments (fig. 1A). The tension decreased markedly in two of the three experiments (*b*, *c*), but only slightly in the third (*a*). The greatest decrease in tension

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occurred in the muscle which had the largest initial tension applied, and the least decrease occurred in the experiment with the smallest initial tension. These results suggest that the rate of decline is a function of the initial tension applied

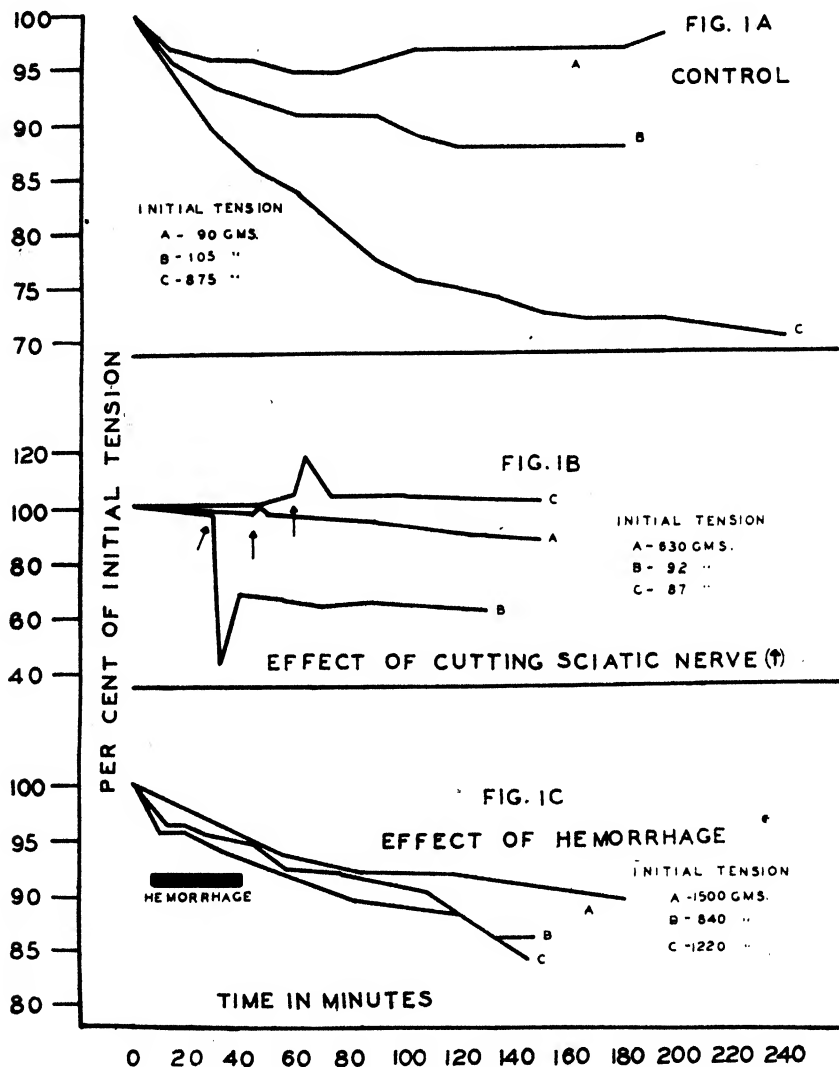


Fig. 1. Tension changes in the gastrocnemius muscle of the anesthetized dog. Changes in tension are expressed as per cent of initial tension applied to the muscle. A, control; B, cutting sciatic nerve; C, effect of hemorrhage.

to the muscle. It is possible that the decrease in tension is due to a stretching of the connective tissues which is sufficient to stretch them beyond the limits of their elasticity, thereby allowing the muscle to elongate passively.

It was thought that if the gastrocnemius muscle of the anesthetized dog pos-

sessed tonus it could be detected by cutting the sciatic nerve and observing any changes of tension which might occur. Three experiments of this type were performed (fig. 1B). Two of these resulted in no change in the downward slope of the tension curve (*a*, *c*). The contraction of the muscles, indicated by the peaks, show that the muscles were irritable. Curve *b* exhibits a sharp decrease in tension upon severing the sciatic nerve, but the tension recovers somewhat a few moments later. Such a curve is difficult to interpret as a tonus change. In view of the character of curves *a* and *c*, it is likely that the sudden decrease in tension in curve *b* is an artifact.

The hemorrhagic method of producing shock has been extensively utilized in this laboratory. Therefore, it was desired to study the effect of hemorrhage on muscle tonus. Three experiments were carried out in which the blood pressure was reduced to 50 mm. Hg by bleeding and maintained at that level for one or two hours (fig. 1C). Hemorrhage did not influence the progressive decrease in tension in any of these three experiments.

From the results of these experiments we conclude that the dog anesthetized with morphine and sodium barbital does not exhibit muscle tonus. The occurrence of shock in such animals then cannot be explained by a failure of a venopressor system actuated by loss of muscle tonus. Since the loss of muscle tonus could not be an initiating or contributing factor in the genesis of shock under these conditions, the present interpretation of changes in intramuscular pressure in the unanesthetized animal in the shock state must be viewed with reserve.

#### SUMMARY

Changes in muscle tension of anesthetized dogs were followed by means of an optically recording isometric muscle lever. Control experiments showed a progressive decline in tension which might be correlated with the initial tension applied to the muscle. The downward slopes of the tension curves were not materially affected by cutting the ipsilateral sciatic nerve or by hemorrhage. It is concluded that the anesthetized dog does not possess skeletal muscle tonus and, therefore, loss of tonus cannot be an initiating or contributing factor in shock.

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# HYPERTENSIVE EFFECT OF L-DOPA AND RELATED COMPOUNDS IN THE RAT<sup>1</sup>

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In 1939 Holtz and Heise (1) described an enzyme in mammalian tissues which is able to convert the amino acid, dihydroxyphenylalanine (dopa), to the powerful pressor substance, dihydroxyphenylethylamine (hydroxytyramine). In the presence of oxygen the resulting amine is quickly destroyed, and it is for this reason, presumably, that an injection of dopa into normal dogs or cats does not produce a marked rise of blood pressure. In 1941, however, Bing and Zucker (2) noted that if dopa were injected intravenously into cats whose kidneys were made partially ischemic by constricting the renal arteries, a rise of blood pressure would occur. This phenomenon is allegedly due to the decreased oxygen tension in the kidneys, whereby the oxidases destroying the amine are inhibited. Dopa decarboxylase, on the other hand, acts in the absence of oxygen.

These experiments suggested to Oster and Sorkin (3) that the response of the blood pressure to an injection of dopa might be a measure of intrarenal oxygen tension. Consequently, they gave from 80 to 450 mgm. of l-dopa intravenously to normal and hypertensive human subjects and observed that the rises of blood pressure were greater and more prolonged in those patients with essential hypertension. This, they concluded, may have resulted from defective renal deamination due to inadequate blood supply of the kidney parenchyma.

If the blood pressure response to this amino acid is truly a measure of renal ischemia, it would be of considerable clinical importance. Before undertaking injections of this substance in other hypertensive disorders, it seemed advisable to study the phenomenon further in the experimental animal. Schroeder (4) reported that normal rats also respond to dopa with a rise of blood pressure; therefore it was decided to utilize this animal for investigation.

*Results on normal rats.* Blood pressures were taken at intervals of 1 to 5 minutes on unanesthetized rats by the tail plethysmograph method of Williams, Harrison and Grollman (5) using a 25 mm. tail cuff. The injection of 3 mgm. of dopa intraperitoneally in 10 normal rats weighing 200 to 280 grams produced rises of 24 to 48 mm. Hg in the systolic blood pressure (mean = 32 mm.). Following the injection, the blood pressure did not begin to rise for 5 to 8 minutes, reached its peak in 10 to 20 minutes and persisted for 10 to 30 minutes. Five milligrams were given to 7 rats, and produced greater rises ranging from 52 to 85 mm. (mean = 61 mm.), but of the same duration. Doses of 8 to 10 mgm. were usually fatal. During the hypertension the animals became restless, moist and had rapid heart rates.

<sup>1</sup> Supported by a grant from the John and Mary Markle Foundation.

In three anesthetized rats with carotid cannulae attached to a Hurtle manometer, an intravenous injection of dopa produced the same characteristic response and the same rise, showing that the hypertensive effect is not concerned with the route of administration. The intraperitoneal dose per kilogram of body weight (10–15 mgm.) was similar to the largest intravenous dose used on man by Oster and Sorkin, although the rises of blood pressure in the rat were higher. Several times this dose per kilogram has been given to normal cats or dogs with negative results.

It seemed strange that the rat should show such a pressor response to this amino acid as opposed to the dog or cat unless the rat's tissues contained more dopa decarboxylase. We have not, however, been able to detect this enzyme in any rat tissues, although we have demonstrated it readily in the kidneys of guinea pigs, rabbits, monkeys and man (6). Holtz and Credner (7) have recently found this enzyme in the liver and kidneys of rats, but in very minute amounts.

*Bilateral nephrectomy.* Both kidneys were removed from five animals and their responses were tested one and two days postoperatively. The rise of blood pressure to 3 mgm. of dopa intraperitoneally was greater (mean = 41 mm.) and considerably more prolonged (over one hour) than in the intact animals. In two rats the blood pressure showed no tendency to fall after 2 hours, demonstrating that the kidneys are concerned with the destruction or elimination of the pressor substance, *but are not essential for the pressor response.*

*Adrenalectomy or hypophysectomy.* Bilateral adrenalectomy was performed on five animals, and on the first to third postoperative day it was found that the response was abolished in all but one animal in which the injection produced a sharp rise followed by death. This was interesting in view of Blaschko's statement that dopa might be the precursor of epinephrine (8). We therefore repeated the experiment in five animals, but this time the rats were given 1 mgm. of desoxycorticosterone daily for two weeks after the adrenalectomy in order to maintain them in good condition. All of these animals responded with rises of blood pressure characteristic of normal rats; so that the adrenal medulla is apparently not essential for the occurrence of this pressor action. Two rats also gave normal responses several weeks after total hypophysectomy.

*Hypertensive rats.* Five rats were made hypertensive by a partial constriction of the left renal artery, and the systolic blood pressures stabilized between 150 and 180 mm. (preoperative range 105 to 126 mm.). The injection of 3 mgm. of dopa produced the same responses as observed in normotensive animals.

*Related compounds.* In an attempt to shed further light on the mechanism of this pressor response to l-dopa, the action of catechol and chloracetocatechol was studied<sup>2</sup>. Both of these compounds produce pressor responses in the intact cat, although the chloracetocatechol is believed to exert its effect by stimulation of the medullary centers (9). An injection of 5 mgm. of catechol produced rises of blood

<sup>2</sup> These compounds, as well as the l-dopa were generously supplied by Dr. Gordon Alles, Pasadena, Calif. The dl-dopa was obtained from the Amino Acid Manufacturers (Dr. Max Dunn), Los Angeles. Fournau 933 was given to us by Doctor Luduena of Stanford University School of Medicine.

pressure of the same magnitude and duration as 3 mgm. of l-dopa. Chloraceto-catechol resulted in mixed responses, the rises in blood pressure being followed by sharp falls. Because catechol and its derivatives are organic reducing agents, the action of a totally unrelated reducing agent, acetaldehyde, was observed. It was shown by Nelson (10) that this substance has a pressor action on cats and is potentiated by cocaine. A dose of 3 mgm. (12 mgm./kgm.) produced an average rise of 47 mm., and 5 mgm. an average rise of 66 mm. The hypertensive effect did not begin for 23 to 31 minutes and persisted over half an hour. The characteristics of the blood pressure response were very similar to those which follow the injection of l-dopa.

Cocaine (20 or 40 mgm./kgm.) was given to 6 rats, and Fournau 933 (10 or 20 mgm./kgm.) to 3 rats, and after 30 to 50 minutes the response of the blood pressure to l-dopa was determined. No alterations of the pressor response were noted.

*Optical rotation.* Unfortunately, we have not discovered why l-dopa raises the blood pressure. Is the amino acid itself a pressor substance in the rat, or is it converted to hydroxytyramine, sympathin, epinephrine, or some other catechol derivative? It was found that about twice the amount of dl-dopa must be injected to produce the same rise as l-dopa on the same animal (12 expts.). Is this because the d-form is not a pressor substance or because, as we already know, only the natural l-form is enzymatically converted to the amine? That the two preparations contained within 10 per cent of the same amount of dopa per milligram was shown by comparing colorimetrically the yield of hallochrome and of melanin formed by addition of fresh potato juice to 100 gamma of each at a pH of 8.0.

*Perfusion experiments.* The rise of blood pressure in unanesthetized rats following the injection of hydroxytyramine (dihydroxyphenylethylamine) or of epinephrine is very short and totally unlike the effects of l-dopa. Furthermore, the hind quarters of a rat, perfused with saline, show intense vasoconstriction to a minute amount of hydroxytyramine but not to 50 times the amount of l-dopa. These observations suggest the possibility that l-dopa might be converted to a related amine *in vivo*, but the fact that catechol and acetaldehyde in the same concentration produce identical responses, coupled with the apparent absence of l-dopa decarboxylase in rat's tissues, speak against this view. The mechanism may be dependent upon the reducing properties of the catechol ring, or upon an ephedrine-like action in blocking those enzymes responsible for the destruction of epinephrine (11).

#### SUMMARY

The amino acid dihydroxyphenylalanine ("dopa"), given intraperitoneally or intravenously in a dose of 12 mgm./kgm., causes a marked and sustained rise of the blood pressure of the rat. Similar rises are observed after hypophysectomy, or in renally hypertensive rats. Adrenalectomy abolishes the response initially, but not after the animals are maintained for two weeks with desoxycorticosterone. Bilateral nephrectomy enhances the magnitude and duration of the response.

L-dopa differs in action from its corresponding amine (hydroxytyramine) both

*in vivo* and *in vitro*. It could not be shown conclusively whether l-dopa exerts its pressor action by decarboxylation to a related amine, or by virtue of its reducing properties, or as a competing substrate for those enzymes which destroy epinephrine. Catechol and acetaldehyde produce similar pressor responses in comparable doses, while chloracetocatechol results in mixed responses. The natural l-dopa is twice as active as the dl-dopa.

The blood pressure response of rats to l-dopa cannot be used as a measure of the decarboxylase content of the tissues, nor as an indicator of intrarenal oxygen tension. Unless it can be shown that dopa has an entirely different mechanism of pressor action in man, it cannot be assumed that clinical responses to its intravenous injection bear any relationship to the functional state of the kidneys.

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## HEMORRHAGIC-HYPOTENSION SHOCK IN LOCALLY ANESTHETIZED DOGS

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A hemorrhagic-hypotension procedure has been developed and standardized to the extent that it can be relied upon to induce an irreversible and hence a fatal shock state with reasonable consistency in barbitalized and chloralozanized dogs (1, 2). Utilizing this procedure, with modifications, similar studies were extended to dogs subjected only to local anesthesia (at operative sites) for several reasons. *a.* Hemorrhagic shock is clinically manifest in humans in both the anesthetized and unanesthetized state; hence, comparative information concerning animal reactions under local and systemic anesthesia should prove useful. *b.* On the basis of the reports of Andrews (3) and others, the suspicion has arisen that the development of shock in experimental animals may be facilitated if not actually eventuated by the employment of barbiturate anesthetics. Although the use of the latter when properly administered, is adequately defended in a recent review article (4), it seemed that a comparative program of this nature might uncover additional pertinent information. *c.* In barbitalized dogs it is impossible to detect the onset of irreversible changes during the advanced stages of the hypotension period. It was hoped that this might be more readily recognized in the absence of general anesthesia. *d.* A program of advanced shock studies in locally anesthetized dogs could not be undertaken until the applicability of this shock producing procedure, previously standardized on barbitalized animals, was evaluated.

Operative procedures were essentially negligible, consisting of cannulation of a femoral artery and vein after the area concerned had been anesthetized with novocaine. Blood was withdrawn into a Liquaemin solution.<sup>1</sup> To circumvent the possibility of injecting large particles (clots) which might have formed before the blood and Liquaemin had been thoroughly mixed, all withdrawn blood was filtered through fine cotton gauze just prior to reinfusion.

*Series A. Experiments—Results and Discussion.* The standardized technique recommended by Huizenga, Brofman and C. J. Wiggers (2) was followed in this series. In brief, an initial period of moderate hypotension (50 to 60 mm. Hg) was established by rapid bleeding and maintained for 90 minutes, usually by additional careful bleeding; thereupon a period of drastic hypotension (30 to 35 mm. Hg) was induced and continued for an additional 45 minutes when possible. Following these 135 minutes of hypotension, all withdrawn blood was re-infused.

Thirteen dogs, under local anesthesia only, served as experimental animals

<sup>1</sup> We are indebted to Roche-Organon Inc., Nutley, New Jersey, for the Liquaemin used in this study.

in this series. It was soon apparent that the recommended intensity and duration of hypotension was unnecessary. Premature deaths occurred in 3 animals before reinfusion measures could be instituted. Only 2 dogs were permitted to endure the full 135 minutes of low blood pressure; in the 8 remaining, the hypotension period was prematurely terminated in the late stages whenever cardio-respiratory failure seemed imminent. All 10 of these animals, nevertheless, died shortly thereafter (see fig. 1A).

Blood pressures taken immediately after reinfusion were as follows: 55, 115, 80, 115, 110, 90, 90, 85, 90 and 100 mm. Hg. Instead, however, of connoting a favorable prognosis, the reasonably satisfactory recovery of blood pressure

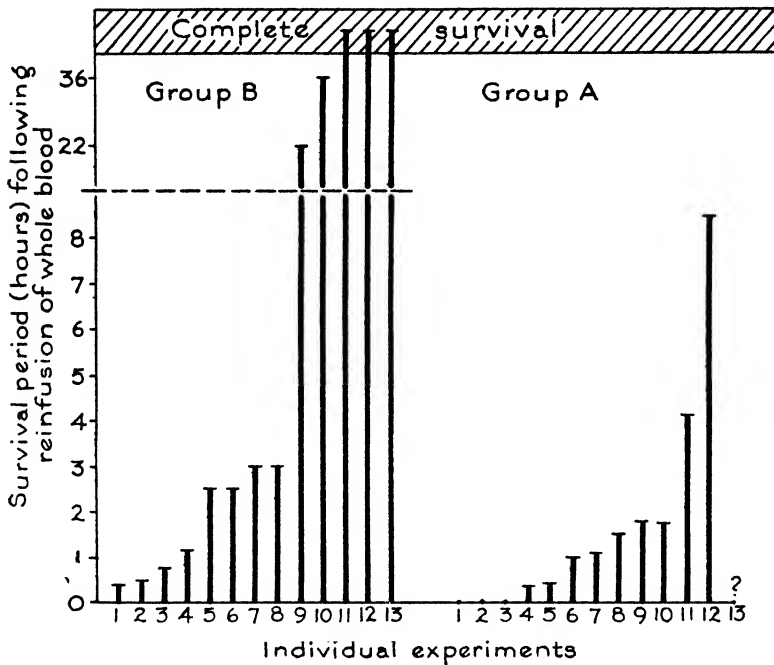


Fig. 1. This depicts the variability in the length of survival of dogs submitted to 135 minutes of moderate and drastic hypotension (group A) and of those subjected to a single 90 minute period of hypotension at 40-45 mm. Hg (group B).

merely masked the truly critical state which had already supervened. Shortly thereafter, the blood pressures declined rather rapidly until death ensued. It is reasonable to assume that the cardiac output also diminished rapidly during the post-reinfusion period, as has been demonstrated in barbitalized dogs under like conditions (5).

The bleeding rate was rapid (50-75 ml./min.) until arterial pressure had declined to within 10 to 15 mm. Hg of the desired hypotensive level, whereupon the rate was greatly retarded. The latter accounts for the great variation in bleeding rate seen in table 1. The values for the net-total hemorrhage (table 1) include additional withdrawals and/or small reinfusions required to maintain

the established hypotension level. Since greater volumes of blood can be withdrawn without fatality from animals under local anesthesia than from those systemically anesthetized (6) we anticipated considerably greater hemorrhaging would be required to establish and maintain arterial hypotension of equivalent intensity in this series of animals. The net-total hemorrhage values reported for barbitalized dogs by Huizenga et al. (2), however, do not support such a contention. If any distinction were made, it would favor equivalent or smaller total-hemorrhages per kilogram of body weight in our dogs. Considering the individual variability in total-hemorrhage values, it is easy to understand why irreversible shock can be reproduced with greater consistency in different animals by bleeding to establish and maintain low arterial pressure for a given interval than by bleeding a certain percentage of total body weight or of the presumed circulating blood volume.

In further comparing our findings with those reported for barbitalized dogs (2, 5) under like circumstances, the following additional facts stand out: *a.* The dogs in this series were less able to withstand the 135 minutes of *moderate* and *drastic* hypotension; in fact, substantial reinfusions were required to sustain life in the advanced stages of hypotension in all but 2 dogs. *b.* "Precipitant"

TABLE 1

Exper. series.....	NET-TOTAL HEMORRHAGE ML./KGM.			BLEEDING RATE TO ESTABLISH HYPOTENSIVE LEVEL IN ML./MIN./KGM.		
	A	B	C	A	B	C
Range.....	16-54	28-64	29-63	2.00-6.65	1.35-4.60	2.00-6.40
Average.....	36.5	43	45	3.68	3.05	3.20

shock deaths were more frequent in these dogs (70 per cent as against 40 per cent in Huizenga's barbitalized dogs). *c.* Again, the mortality in the latter was *ca* 70 per cent, whereas in this investigation it was 100 per cent.<sup>2</sup> *d.* The pathological findings at autopsy seemed more intense and extensive than those observed by one of us in a similar series of experiments on barbitalized dogs (5).

Chiefly on the basis of the above comparative findings, the following conclusions are reached: *a.* The incidence of irreversible shock seems to be accelerated and the condition more severe in the locally anesthetized as compared with the barbitalized dog. *b.* The procedure utilized for producing shock is unnecessarily severe for animals under local anesthesia.

The autopsy findings deserve special comment. In the 3 dogs which died prematurely in the advanced stages of hypotension, the intestinal mucosa, on

<sup>2</sup> In personal correspondence, Dr. Samuel Middleton of the University of Chile relates that he repeated the *moderate* and *drastic* hemorrhagic-hypotension procedures on 4 barbitalized dogs and that every one of them survived. It will be interesting to examine the percentage mortality when he completes his series of experiments. Huizenga, Middleton and one of us (HCW) collaborated on similar experiments when Middleton was in this country; hence, any dissimilarities in his results are probably not attributable to technical variations.

gross inspection, appeared but slightly congested. Upon inspection under the microscope, however, the capillaries and venules were seen to be extremely engorged and every field examined contained numerous extravasated red cells. Presumably small amounts of plasma had also leaked out of the distended capillaries. There is little question that the capillaries had been irretrievably damaged. The absence of large amounts of bloody tinged fluid in the lumen is unquestionably related to the co-existent low arterial blood pressure. In this connection, it will be recalled that Fine and Seligman (7) failed to notice any acceleration of the disappearance rates of radioactive plasma proteins from the circulation in the untreated advanced stages of hemorrhagic-hypotension. Cardiac output was also quite stable during this phase in barbitalized dogs (5).

When, however, the blood pressure is elevated by reinfusion after a protracted hypotension period (as in 10 of our dogs), the gut at autopsy not only revealed a grossly hemorrhagic mucosal wall, but was filled with fluid containing much free blood and probably all plasma constituents. These hemorrhages on occasions extended to the stomach mucosa, large intestinal mucosa and to the pancreas. Fine and Seligman similarly reported that any infusion in the advanced hemorrhagic-hypotension state leads to an acceleration of the disappearance rate of plasma proteins from the circulation. It is reasonable to assume that the resulting increased blood pressure and blood volume are responsible for increased loss of whole blood through capillary walls whose integrity had been previously impaired. The progressive post-reinfusion diminution of cardiac output seen in barbitalized dogs was undoubtedly associated with this great leakage of cells and plasma into the intestinal lumen and perhaps in other regions.

*Series B. Experiments—Results and Discussion.* The combination of *moderate* and *drastic* hypotension as previously designated proved unnecessarily severe for standard employment as a method of producing shock in dogs locally anesthetized. The method was, therefore, modified and tested in 13 dogs, using novocaine at the site of cannulation. Using essentially the same bleeding procedures, these animals were subjected to a single 90 minute period of hypotension (40–45 mm. Hg) following which all withdrawn blood was readministered. The less strenuous demands of this procedure are indicated *a*, by the generally longer post-reinfusion survival times (compare fig. 1A and 1B); *b*, by the reduced severity of pathological disturbances seen at autopsy, and *c*, by the fact that 3 dogs survived indefinitely as against no survivals in the A series. The method is, therefore, recommended as more favorable and more convenient for the production of hemorrhagic shock under these conditions.

The arterial blood pressures immediately after reinfusion were as follows: 50, 60, 65, 75, 75, 90, 90, 90, 95, 95, 110, 90 and 175 mm. Hg. The last 3 values were obtained in the survival dogs. Though the general level is decidedly lower than in the series A dogs, the post-reinfusion survival times were generally longer than in the previous series. Thus, the fallacy that blood pressures can serve as a criterion of the state of the animal in these conditions is again emphasized. The net-total bleeding volumes were slightly greater than in the A series.



It was not possible, as had been hoped, to detect the onset of the irreversible changes which develop during the hypotension period. A definite physiological sign, however, was observed in the terminal portion of the hypotensive period, which does indicate that severe irreversible changes have developed. This indication was observed in all dogs in the A series and in the 10 non-survivals in this B series, as well as in barbitalized dogs under similar conditions (5). In this reaction, there is a *persistent* tendency for the blood pressure to decline spontaneously below the established hypotensive level; *persistent* in that blood pressure begins to fall again toward critical levels even though the hypotensive level be momentarily regained by repeated infusions of blood and/or saline. This reaction did not occur in the survival dogs. It must not be inferred that such a reaction always occurs when irreversible changes have developed (i.e., we have failed to recognize it in several shock animals of a later series). It is quite likely the sign would have appeared in the 3 survival animals and that these also would have succumbed in shock, had the hypotension period been slightly extended beyond the 90 minute limit. In fact, irreversible shock can probably be produced without fail if the hypotension period is continued until spontaneous and persistent reduction of blood pressure is recognized. It is important to recognize that whenever this reaction occurs, none of the remedial measures heretofore employed to treat hemorrhagic shock conditions can provide more than an apparent temporary benefit to the animal. Furthermore, it is useless and unwise to continue the shock producing procedure beyond this point.

Apparently the 3 survival dogs were better endowed with compensatory powers; or perhaps the latter were more efficiently conserved during the initial stages of the hypotension period. It is significant that the blood pressure persistently tended to rise above the established level throughout the entire low pressure period, thus necessitating repeated withdrawals of blood. In addition, as revealed by specific gravity studies, the blood continued to dilute during most of the hypotension period; this did not occur in the non-survival animals, which may partially account for the better compensatory adjustments in the 3 survival animals.

*Series C. Experiments—Results and Discussion.* This series of experiments was undertaken to determine the duration of hemorrhagic-hypotension of the intensity employed in B experiments which can be endured without the development of irreversible shock. As there is no known criterion to indicate the moment at which total reinfusion will just save a given animal, the following procedure was adopted and tested in 10 animals with only local anesthesia. With similar bleeding rates (table 1, series C) an arterial blood pressure of 40 to 45 mm. Hg was established and maintained for an abbreviated period of 60 minutes, whereupon all withdrawn blood was reinfused.

The immediate post-reinfusion blood pressures were definitely more satisfactory than in the A and B series (120, 140, 140, 125, 130, 150, 110, 115, 120 and 150 mm. Hg). It is again pertinent to relate that the last value (150 mm. Hg) was obtained in the *only* dog of this series which did not survive. This animal died of shock, exhibiting the spontaneous decline of blood pressure just

prior to the completion of the hypotension period. His post-reinfusion survival time exceeded that of most *A* and *B* dogs and the typical intestinal and cardiac hemorrhages were present but mild. The remaining 9 animals survived indefinitely.

In view of the high mortality in the *B* group and high percentage of survivals in this series (9 of 10), it seems that the *irreversible* state usually develops during the last 30 minutes of the 90 minute hypotension period. In most instances, it appears to have become recognizable within the last 15 minutes of this period.

It is possible that the hypotension period might be slightly extended beyond 60 minutes with the expectancy of many survivals; the 60 minute period, however, is sufficiently severe to permit the evaluation of the efficiency of various blood and plasma substitutes in severe hemorrhagic conditions when administered in place of or along with withdrawn blood at the end of the hypotension period.

**DISCUSSION.** It is claimed by Moon and his co-workers (8) that hemorrhaged animals do not die of shock but rather from moribund or comatose conditions which develop as a result of hemorrhage. In reference to this, it is pertinent to relate that none of the series *A* or *B* dogs were either moribund or comatose until the dying moments late in the post-reinfusion period. Even in the advanced stage of hypotension, reflexes were decidedly active and, in general, the dogs responded to manipulations designed to attract their attention. Those which did not succumb prematurely appeared to be in reasonably good physical condition when released from the supine position; in fact, some were amazingly energetic. From their general attitude, it was usually impossible to predict which would or would not survive. However, it was assumed that those which had exhibited the spontaneous decline of blood pressure in the hypotension period and which had vomited and/or passed bloody fluid fecal material would not survive indefinitely. Predictions based upon these criteria proved correct in every instance.

In further disclaiming that shock can be produced by uncomplicated hemorrhagic procedures, Moon (8) differentiates between conditions observed in shock dogs which he failed to recognize in hemorrhaged dogs. It is interesting that most of his differentiating shock characteristics were common to our hemorrhaged dogs. To mention a few: *a*, the intestinal capillary endothelium was undoubtedly rendered permeable to colloids; *b*, pulmonary edema was noted on occasions; *c*, diarrhea (bloody) and vomiting were common occurrences; *d*, blood reinfusions were rarely effective; *e*, blood specific gravity, though initially reduced, began to increase before reinfusion was instituted and greatly surpassed control values in the post-reinfusion period; *f*, severe capillovenous congestion was an obvious feature; *g*, petechiae in intestinal mucosa were always present and frequently so in the heart and in various skeletal muscular regions, and finally *h*, visceral ischemia was never noticed. Though admittedly naive to assert that shock always prevails when dogs succumb to large hemorrhages, it is important to recognize that uncomplicated hemorrhages can readily precipitate conditions in which all the essential complications of shock are present.

# THE MECHANISM OF EPINEPHRINE BRADYCARDIA AND SHOCK IN YOUNG ANIMALS

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It is generally accepted that the cardiac slowing which follows the injection of epinephrine is due to a vagus action. Whether it is a depressor reflex from the aortic arch and the carotid sinus or is the result of the direct action of the drug on the vagus center, it is certain that this slowing is absent when the vagus nerve is cut (1, 2). In newborn animals the central control of the heart by the vagus is absent for 12 to 15 days after birth (3, 4, 5). Furthermore, tonic vagus excitation is absent until the puppy opens his eyes (5) and cardiac reflexes from stimulating the carotid sinus, aortic arch and upper laryngeal region remain undeveloped for some time. These considerations led to the expectation that epinephrine bradycardia would be absent in the newborn.

In adults large doses of epinephrine lead to death within a few minutes (6, 7, 8, 9). This action is said to be due to a tonic stimulation of the drug on the vagus center (10). The newborn animal is very resistant to histamine (11, 12) and it is generally accepted that shock-producing agents are less effective in the newborn than in the adult. These considerations lead to the idea that the newborn may be more resistant to large doses of epinephrine than is the adult.

The purpose of the present study, therefore, is to ascertain the peculiarities of epinephrine action on the cardiovascular system of very young animals.

**METHODS.** The investigation was carried out on puppies during the first 90 days *post partum* and on rabbits during the first 40 days. Parallel control experiments were done on adult animals. In all, 75 experiments were done on dogs and 58 on rabbits. All injections were made into the external jugular vein. The puppies' blood pressures were recorded with a mercury manometer from the carotid artery. These animals were under ether. The rabbits were not narcotized. Their heart beat was visualized by thrusting a needle through the thoracic cage into the heart wall. The heart beat and respirations were recorded with a signal magnet. The animals were brought to autopsy with careful control of lung changes. Microscopic observations were made in the laboratory of Prof. F. I. Pojarisky by Dr. R. V. Erleksova, to whom we are grateful for her data on histological control. The epinephrine used came from the Moscow Factory for Endocrine Preparations.

**EXPERIMENTAL RESULTS.** Preliminary experiments on adult dogs confirmed the generally accepted idea as to the vagal origin of cardiac slowing after epinephrine. When small doses of epinephrine (12.5  $\mu$  g./kilo) were administered to puppies the heart speeded up and the blood pressure rose (see fig. 1a). A

slightly larger dose ( $25\mu$  g./kilo) gave rise to a marked bradycardia accompanied by a rise in blood pressure (fig. 1b). This last finding is contrary to the results of Thiele (13) who denies that epinephrine may produce bradycardia in very young animals.

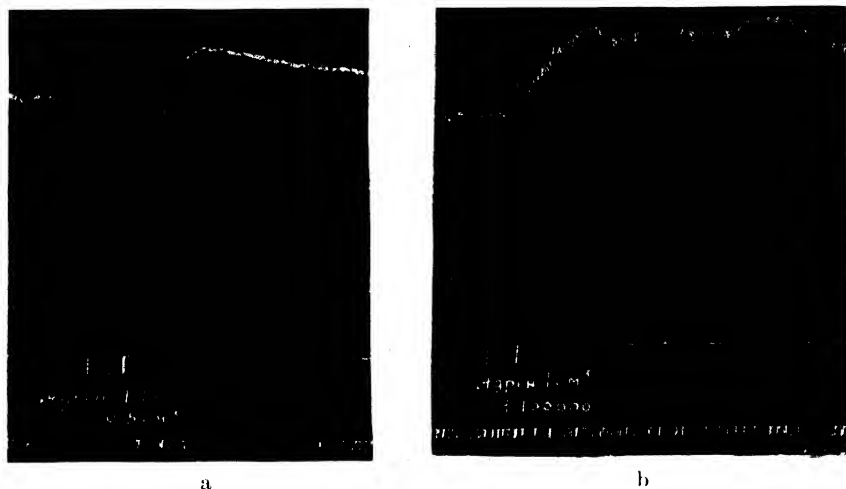


Fig. 1. The effect of an injection of epinephrine on the blood pressure of a 6 day old puppy weighing 400 grams. (a) Tachycardia with a rise in blood pressure after injecting 12.5 micrograms per kilo. (b) Bradycardia with rise in blood pressure after injection of 25 micrograms per kilo.

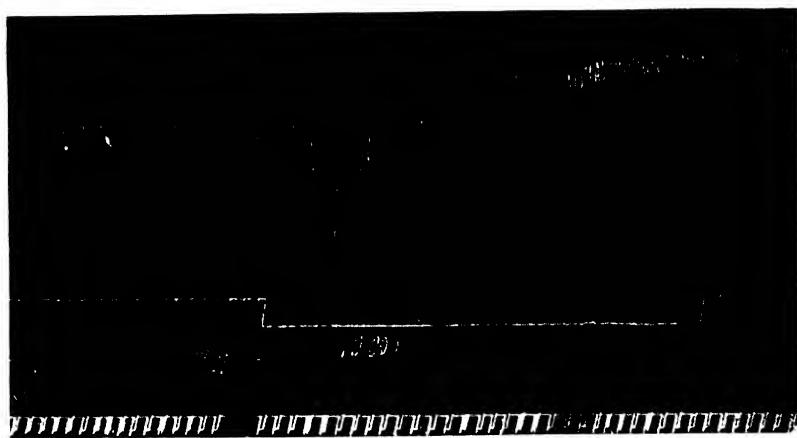


Fig. 2. Effect of a large intravenous injection (0.43 mgm. per kilo) of epinephrine on a 6 day old puppy weighing 350 grams.

When the dose of epinephrine is increased (0.43 mgm./kilo) bradycardia is sufficiently marked to prevent the rise in the blood pressure seen with smaller doses and even to produce a prompt fall in the arterial tension (fig. 2).

Since the cardiac slowing often occurs without any preliminary rise in arterial pressure it seems doubtful that the slowing could be due to the stimulation of

reflexes from the carotid sinus and aortic arch. To make this point sure the experiment was repeated on puppies whose vagi had been cut. Bradycardia ensued in all cases (see fig. 3) and in many cases the records after the vagi were cut were exact replicas of those obtained before the vagi were cut. It is clear then that the bradycardia produced in very young animals by epinephrine is not due to a reflex through the vagus as is the case in the adult.

It has been shown that the perfused heart of the puppy responds to epinephrine as does the perfused heart of the adult, with an increase in rate and strength of beat (16). It is clear then that the action of epinephrine to produce bradycardia in puppies is not due to its influence in stimulating the vagal endings within the heart as has been said (14, 15).

It has already been reported (5, 16) that the sympathetic system has a very strong tonic activity in young puppies and that sympathectomy gives rise to marked slowing of the heart and to a disappearance of epinephrine bradycardia.

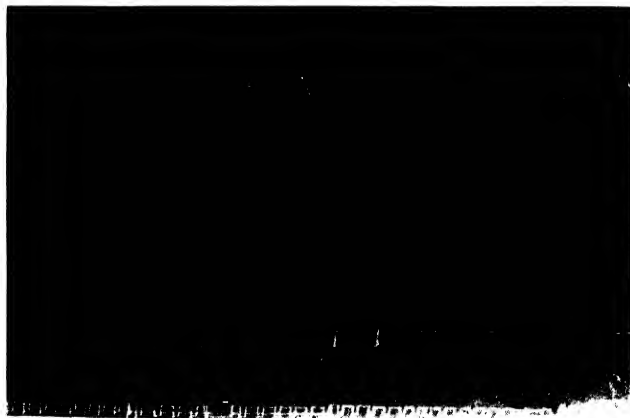


Fig. 3. Effect of a large intravenous dose of epinephrine (2.6 mgm. per kilo) on a 6 day old puppy weighing 380 grams after both vagi had been cut.

The evidence points to the idea that the sympathetic innervation of the heart is responsible for epinephrine bradycardia. Small doses of epinephrine increase the sympathetic action upon the heart giving rise to acceleration. Large doses inhibit sympathetic action upon the heart and leave the heart to beat by a rhythm originating in the heart, uninfluenced by sympathetic accelerating influences. The manner in which this inhibition occurs is best understood in terms of the concept of the Wedensky-Ukhtomsky school. Peripheral volleys to the heart are cut off through over-excitation of the sympathetic neurones.

This reaction can be thought of as expedient in the sense that it prevents an undue rise in blood pressure in the same manner as do the carotid sinus and the aortic arch reflexes which are absent at this time (17, 5, 18). It may have a similar function to the lung release reflex (19, 20).

With moderate doses (2-5 mgm./kilo) the reaction is reversible and the animal usually survives. Doses larger than 4 mgm. per kilo usually give rise to death through "epinephrine shock". The immediate symptoms are a slow

heart (60–40 beats per min.), a slow respiration (2–3 per min.) and a very low blood pressure (20–30 mm. Hg) (fig. 4). Death does not ensue until 30 to 90 minutes after the injection. This is in contrast to the almost immediate death which occurs in the adult from an overdose of epinephrine. The course of exitus is not very different from that described for anoxic shock in the newborn (21).

If small doses of epinephrine are administered during the early development of epinephrine shock, there results a temporary rise in blood pressure (fig. 5).

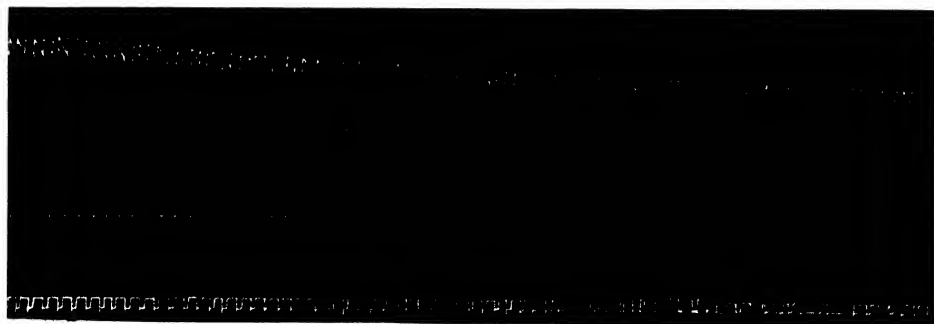


Fig. 4. Effect of a massive dose of epinephrine (over 4 mgm. per kilo) giving rise to a slow heart and a decline in blood pressure leading to death in 30 to 90 minutes (epinephrine shock).

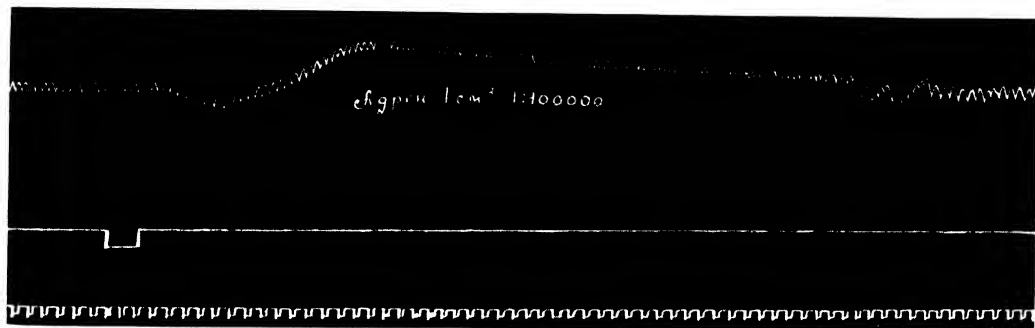


Fig. 5. Effect of a small dose of epinephrine given shortly after production of epinephrine shock.

This response is entirely absent if the same dose is given 10 to 20 minutes after the initial injection. "Epinephrine shock" is interpreted as a sympathetic paralysis involving the cardiac and vasomotor nerves. The slow heart beat continues from an autonomic rhythm.

The reactions of rabbits are similar to those of dogs. Small doses of epinephrine cause acceleration whereas larger doses cause a short stoppage of the heart followed by a resumption of beat at a slow rate. As in the dog the reaction is entirely independent of the vagi and is similar to the reaction which is seen in young rabbits when the head is strongly compressed (22). The latter is an inhibition of sympathetic activity, probably of the Wedensky type.

The action of epinephrine on the young rabbit is reversible with doses of 0.5 to 5 mgm. per kilo but it is irreversible (epinephrine shock) with doses of 5 to 10 mgm. per kilo. With doses of this size death ensues in 30 to 180 minutes. It takes doses of 20 to 30 mgm. per kilo to kill the young rabbits as quickly as 0.4 mgm. to 1 mgm. per kilo will kill the adult (i.e., within 2-3 min.).

One of the important findings in adult rabbits that have died from epinephrine overdose is pulmonary edema (23 and 24). Careful examination of the lungs of very young rabbits which have died after large doses of epinephrine has failed to demonstrate edema. The tissues of the lungs are capable of becoming edematous because lung irritant gases will produce typical edema (25). The reason that epinephrine fails to produce edema seems to be that the rapid initiation of bradycardia in young rabbits prevents the distention and congestion of the vascular bed of the lung and hence prevents edema from the filtration of fluid out of the pulmonary capillaries.

#### SUMMARY

Puppies less than 45 days old whose vagus centers show neither tonic activity nor reflex excitability respond with cardiac acceleration to small doses and cardiac slowing to large doses of epinephrine. Puppies and young rabbits show these responses after section of both vagi but the slowing does not occur after sympathectomy (16, 5). Epinephrine bradycardia is through sympathetic innervation in very young animals.

Epinephrine bradycardia is reversible if the dose is not too large. If the dose exceeds the fatal dose for adults by eight to ten times, the young animal will die in 30 to 180 minutes. Death in 2 to 4 minutes, characteristic of the adult rabbit, occurs in the young rabbit only if the dose is 20 to 30 times the lethal dose for the adult.

Young rabbits do not show lung edema after epinephrine as do adults, though it may be produced in the young rabbit by lung irritants.

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small in comparison with the changes in volume that occurred with anoxia. These are shown in the segments on the right side of X which marks the beginning of rebreathing. The extent of the maximal expiratory increase in volume in this experiment was 67 cc. Maximal increases in five representative experiments including this one are given in table 1.

In four of these five experiments a fair degree of uniformity is apparent, all of the increases being within the range of 60 to 71 cc. The animal with a smaller increase was more deeply anesthetized. The oxygen threshold for the chest expansion (increased inspiratory tonus) reaction varies between about 16.5 and 12.5 per cent with an average and mode at about 14 per cent.

The pattern of breathing with a diminishing oxygen supply differs in different animals, but the changes have some common features. Figures 2A and 3B show some of the variations. In 2A there was no great change in depth or frequency until the oxygen content of the inspired air reached levels below 10 per cent. Below this there was a progressive increase in rate. At the lowest O<sub>2</sub> content, 7.4 per cent, the respiratory frequency showed a 75 per cent increase over the

TABLE 1  
*Maximal extent of increase in expiratory volume of cats in anoxia*

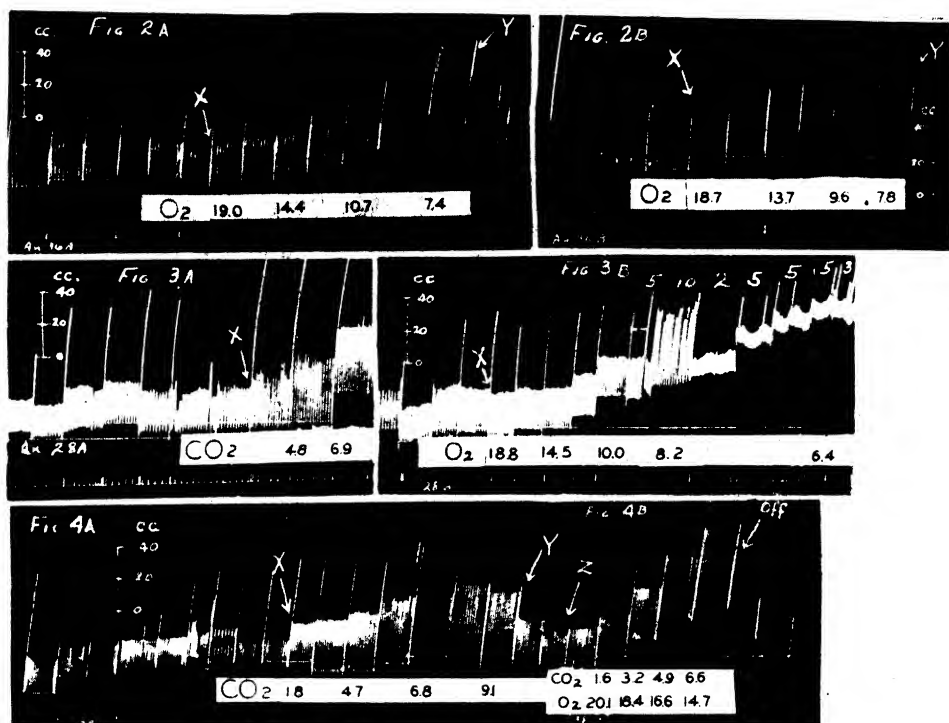
EXPT.	WT.	INCREASED VOL.
	<i>kgm.</i>	<i>cc.</i>
1	2	47
2	2.5	60
3	2.5	71
4	1.9	70
5	2.4	67

control frequency and the depth had decreased by about 10 per cent. The pattern in figure 3B differs in that it shows an intermediary stage of very deep breathing. Following this the respiration became rapid and shallow, the rate becoming three times that of the control while the depth was diminished to one-half that of the control or less.

The rôle of the vagus nerves in the expiratory volume increase in response to anoxia was studied by recording the volume changes in rebreathing trials before and after sectioning the vagi. Figure 2A was recorded before vagotomy and figure 2B after vagotomy. The lowest oxygen percentages in the two trials are comparable, 7.4 per cent before and 7.8 per cent after sectioning the vagi. In this experiment the amount of expiratory volume increase after section of the vagi was 17 cc., as compared with 67 cc. before vagotomy. In the other two similar experiments, also, the amount of expansion after vagotomy was between one-fourth and one-third of the amount recorded in the initial trials with vagi intact. In control experiments with the vagi left intact two successive trials have produced expansions that were approximately equal. The vagal afferent fibers, therefore, appear to have a predominant part in the chest expansion reac-

tion in the cat. In the dog, as will be shown later, the vagus is even more completely in control of this reaction.

The effects of excess carbon dioxide upon the expiratory chest volume in cats vary with the amount of  $O_2$  present. These variations are shown in figures 3 and 4. Before beginning the trial in figure 3A some extra oxygen was added to the



Figs. 2, 3 and 4. Volume changes in the cat.

2A. Rebreathing air between X and Y,  $CO_2$  absorbed. Percentages of oxygen indicated below. Intervals between segments of record, 15 minutes. Time signal, 3 seconds. Base line corrected for temperature changes. 2B. Vagus nerves cut. Rebreathing repeated as in 2A.

3A. Small excess of  $O_2$  (about 25 per cent at end). Accumulating  $CO_2$  whose percentages are indicated below. 3B. Same animal rebreathing air,  $CO_2$  absorbed. Numbers in white, above, give the interval in minutes since the preceding segment. Unmarked intervals are 15 minutes.

4A. Accumulation of  $CO_2$  in the presence of a very large excess of  $O_2$ . 4B. Simultaneous reduction of  $O_2$  and accumulation of  $CO_2$ .

air of the spirometer but not a large excess. An increase in expiratory chest volume began when the  $CO_2$  in inspired air was between 4.8 and 6.9 per cent. The  $O_2$  content was about 25 per cent.<sup>3</sup> Figure 3B, made later, shows the same animal's expansion threshold (about 12 per cent  $O_2$ ) and subsequent response to

<sup>3</sup> The Henderson-Orsat apparatus is not calibrated for accurate determination when absorptions are as great as in this case, but a close approximation could be obtained.

reduction of oxygen tension without excess  $\text{CO}_2$ . Figure 4A shows the response to an accumulation of  $\text{CO}_2$  in the presence of a large excess of  $\text{O}_2$  (near 100 per cent at beginning). There was no increase in expiratory chest volume even at 9.1 per cent  $\text{CO}_2$  in inspired air. Before the second trial with this animal (fig. 4B) the rebreather tank was filled with air, and during rebreathing there was simultaneous diminution of  $\text{O}_2$  and accumulation of  $\text{CO}_2$ . In this case there was an increase in expiratory chest volume beginning, certainly, during the second fifteen minutes (3.2 per cent  $\text{CO}_2$  and 18.4 per cent  $\text{O}_2$ ) if not earlier. These two experiments (figs. 3 and 4) appear to mean that the stimulus of hypercapnia, of itself, does not produce an increase in expiratory chest volume, but that it does increase the sensitivity of the mechanism that responds to low  $\text{O}_2$  tension. It sensitizes the mechanism to such a degree that it becomes active at normal atmospheric  $\text{O}_2$  tensions and somewhat higher. The conclusion that a normal atmospheric tension of  $\text{O}_2$  should excite this reaction when sensitized by  $\text{CO}_2$  loses its surprise when it is remembered that a tonic chemoreceptor respiratory drive at normal atmospheric  $\text{O}_2$  levels has been reported by Watt, Dumke and Comroe (11) and by Bernthal and Weeks (1).

*Dogs.* The expiratory circumference of the thorax of the dog weighing 8 to 10 kgm. has been found to increase under the stimulus of anoxia by a maximum of 10 to 16 mm. at a level 2 in. above the inferior end of the sternum. Figure 5 graphically represents the changes that occurred in three trials on the same animal. The curves marked *Anoxia I* and *Anoxia II*, made before any nerves were interrupted, show girth increases of 12 and 14 mm. respectively.

During  $\text{CO}_2$  hyperpnea in dogs no increase in expiratory girth was found. At the height of the hyperpnea, with powerfully forced expirations, there was a decrease in expiratory girth by about 3 or 4 mm. Reduction was reported by Gesell and Moyer (3) also.

Vagotomy totally eliminated the expiratory girth increase response to anoxia in dogs. In four anoxia tests following vagotomy expiratory girth remained unchanged in two, and diminished by 2 to 4 mm. in the others, one of which is shown in figure 6. The expiratory girth response to  $\text{CO}_2$  is left unchanged by vagotomy.

A test of the rôle of the carotid chemoreceptors in the increased inspiratory tonus response, and a confirmation of the necessity of the vagi are shown in figure 5. The animal, with carotid nerves and vagi intact but exposed, rebreathed the  $\text{O}_2$  of the respirator down to 8.7 per cent (curve *anoxia I*). The expiratory girth increased by 12 mm. After a 30 minute recovery period on air the rebreathing test was repeated (curve *anoxia II*). The oxygen of the tank was breathed down to 9.8 per cent, and this level was maintained by adding  $\text{O}_2$ . The expiratory girth had then increased by 14 mm. While the animal continued breathing 9.8 per cent  $\text{O}_2$ , procaine solution was applied to Hering's nerves. The enlarged chest volume remained unchanged though the respirations became faster and shallower. Then Hering's nerves were crushed (at the point marked *H.N.*), whereupon respiration stopped. The animal was returned to air and revived by a short period of artificial respiration. After a rest of another half-hour on air, rebreathing was again repeated (*-Hering's n. anoxia III*) with an expiratory girth

increase about as great as before. At 10.4 per cent  $O_2$  the increase was 12 mm. The trial was terminated at this point by sectioning the vagi. The circumference immediately dropped back to the control size, again demonstrating that the afferent fibers of the vagus nerves are necessary to the inspiratory hypertonia in the anoxic dog. The difference between the shape of the third curve and that of the others may or may not have resulted from the elimination of the carotid receptors

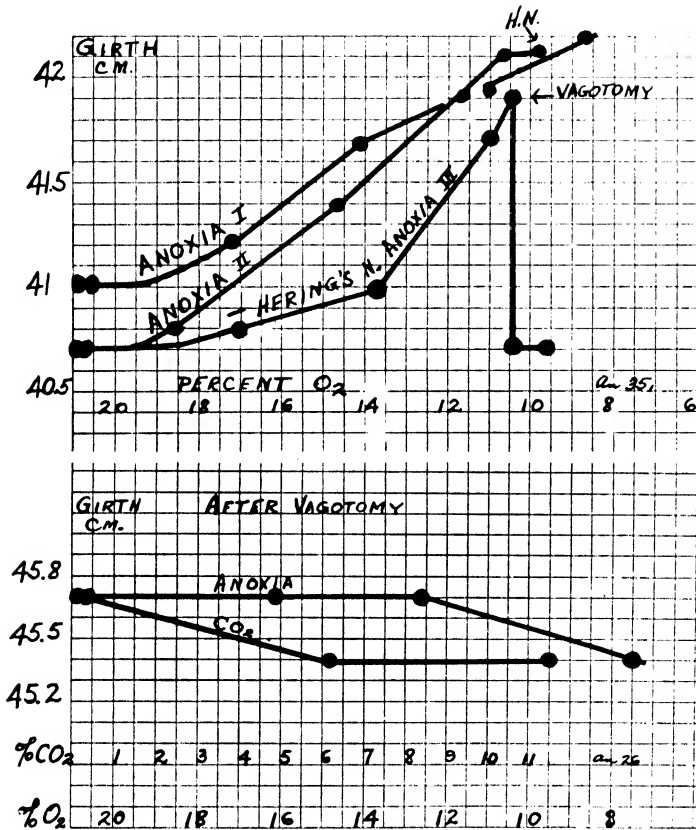


Fig. 5. Changes in the circumference of the dog's thorax resulting from anoxia. Modifications by crushing Hering's nerves and section of the vagi.

Fig. 6. Dog's chest circumference after vagotomy. Effects of anoxia and of hypercapnia.

It could be fortuitous or could have resulted from the effect of two previous anoxic trials upon sensitivity to lesser degrees of anoxia.

**DISCUSSION.** The experiments reveal that the degree of maintained inspiration, or enhanced inspiratory tonus in anesthetized animals under the stimulus of anoxia may amount to three times the depth of a normal inspiration. Upon this large maintained contraction the animal superimposes his respiratory movements. There is evidence that the human chest also enlarges with acute

anoxia (5) and the findings of Hurtado et al. (9) that total capacity and residual air are increased, fit in with this concept. Their finding of diminished vital capacity may be related to the neuromuscular strain of maintaining the enlargement.

When the anoxic stimulus is sufficiently great in the cat, dog, or man (6, 8), the respiratory movements become shallow and rapid. The shallowness of inspiration probably is due to the obvious difficulty of adding a new inspiration to an already deep maintained inspiration. Haldane (6) reasoned that respiratory fatigue caused the short breaths, but the maintenance of a high degree of inspiratory contraction could logically account for the shortness of breaths and the fatigue.

Monge (10) mentions the importance of respiratory training to people at high altitudes. Native Andeans stop at short intervals to take a few deep breaths, then continue on their way with a load the white man would find difficult at low altitudes. If the anoxic enlargement of the human chest approaches that found in the animals, it would seem to follow that a logical way to increase the respiratory exchange and to relieve the tension on the inspiratory musculature would be to exhale more completely. The formation of a habit of forcing expirations should be a great help.

The experiments have shown that an excess of  $\text{CO}_2$  in the oxygen deficient air does not prevent the increase in expiratory chest volume, but it does greatly increase the minute volume of respiration. For this and other reasons reviewed by Gibbs et al. (4)  $\text{CO}_2$  improves the oxygenation of the tissues when the  $\text{O}_2$  tension in inspired air is low. However, there is evidence that the addition of  $\text{CO}_2$  at the expense of  $\text{O}_2$  in a mixture does not increase alveolar  $\text{O}_2$  (12).

Hurtado et al. and other investigators whom they cite (9) have found dilatation of the alveolar spaces, widening of the lung capillaries and an emphysematous appearance of the lungs in animals subjected to anoxemia. They consider these dilatations together with the increase in residual air as being important compensatory adaptive mechanisms. Doubtless this view is correct if life under anoxic conditions is continued long enough for adaptations to mature, but in the acute stage the difficulty of inspiration may more than offset the advantages of larger volume and larger diffusion surfaces.

#### SUMMARY

Methods for plethysmographically recording the volume changes in the cat, and for measuring the chest circumference of the dog during procedures affecting respiration are described.

During the reduction of  $\text{O}_2$  content of inspired air from that of the atmosphere to about 8 per cent by rebreathing the volume of the cat's chest at the end of expiration increases by about 60 to 70 cc. This is three times the volume of a normal inspiration. During a similar diminution of  $\text{O}_2$  in the inspired air of the dog a proportionate increase in expiratory circumference of the chest occurs.

After vagotomy the anoxic increase in expiratory volume in the cat is about one-third as great as with the vagi intact. In the dog vagotomy totally eliminates the anoxic chest expansion. Crushing the nerves from the carotid chemoreceptors has little or no effect upon the reaction.

Hypercapnia appears to sensitize the animal to the low  $O_2$  chest expansion reaction, but in the presence of a large excess of  $O_2$  accumulated  $CO_2$  does not produce an increase in the expiratory volume in cats or dogs. In some animals there is a small reduction in chest volume when the expirations become forceful.

The human expiratory chest volume has been shown to enlarge during dyspnea. The suggestion that respiratory training to exhale more completely may be valuable to people at high altitudes is briefly discussed.

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# THE EFFECT OF SUCCESSIVE FASTS ON THE ABILITY OF MEN TO WITHSTAND FASTING DURING HARD WORK<sup>1</sup>

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Previous reports have<sup>2</sup> indicated that as a result of successive periods of fasting, obese individuals show a metabolic adaptation to this stress by a decreased urine ketone and nitrogen excretion (1). Somewhat similar results have been obtained in the dog (2). This paper will report experiments designed to study this adaptation in greater detail in fasting young men doing hard work. Physiological and psychomotor tests were included to determine whether this adaptation is sufficiently far reaching to influence performance. The data were obtained on four young men who carried out five successive fasts. For the purpose of the present discussion it will suffice to consider the first and fifth fasts.

**CONDITIONS.** The detail schedule is presented in table 1. Total caloric expenditure during the first day of fasting amounted to approximately 4500 calories, 4000 on the second, and 2000 on the third. Work was performed on a motor-driven treadmill at 3.5 m.p.h. and a 10 per cent grade. This rate of work results in an average expenditure of 550 calories per hour in our subjects.

The men were on a carefully planned diet for 3 weeks before each fast. This diet contained 460 grams of carbohydrate, 90 grams of protein, 150 grams of fat and was adequate according to N.R.C. standards in vitamins and minerals.

The first fast occurred in June and the fifth in October. The time interval between the five successive fasts was five to six weeks. The men maintained a standard state of physical condition by walking an hour and a half a day on the treadmill and performing occasional brief runs to "anaerobic" exhaustion during the interim periods. Before each fast, the work done was increased until 4 hours of daily work was performed for two days before the fasting period.

All work was carried out in an air-conditioned suite at 74 to 78° F. and 50 per cent relative humidity. Standard clothes were worn during all observations.

**OBSERVATIONS.** Pulse rates were counted five times during the last 10 minutes of each one-hour work period. Expired air was collected for 20 minutes at rest each morning, and during each one-hour work period when 500 liters of expired

<sup>1</sup> The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the Regents of the University of Minnesota. Important financial assistance was also provided by the Nutrition Foundation, Inc., N. Y., the National Dairy Council, Chicago, the U. S. Cane Sugar Refiners' Association, N. Y., the Corn Industries Research Foundation, N. Y., Swift and Co., Chicago, and the National Confectioners' Association, Chicago. Merck and Co., Inc., Rahway, N. J., provided a supply of pure vitamins. Food materials were supplied by the Subsistence Branch, Office of the Quartermaster General, U. S. Army.

air were collected during the last 15 minutes and blood for sugar determinations was drawn during the last 5 minutes. Twenty-four hour urines (starting at 7:00 a.m.) were collected during the first and second day for urinary nitrogen and ketones.

The battery of psychomotor tests consisted of four tests of speed and co-ordination carried out while the subject was walking on the treadmill, and two tests of muscular strength.

**METHODS.** Expired air was collected with a Krogh type respiratory valve and a compensated gasometer. Gas was analyzed by the direct Haldane method. Pulse rates were counted with a stethoscope. Blood sugar was determined by the method of Folin (3) using cadmium hydroxide as the precipitating agent. Lactate was determined by Edwards' (4) modification of the method of Friedemann and Cotonio. Blood acetone was determined by the method of Barnes and Wick (5). Urine acetone was measured by the method of Van Slyke (6) and nitrogen by the method of Keys (7).

Pattern tracing was used to test eye-hand co-ordination. A visual choice reaction served as a measure of the speed of gross body movements. The ball-pipe test measured speed and co-ordination of arm and hand manipulations, and two-plate tapping test was used as a measure of simple hand speed. Strength was determined by standard hand and back pull dynamometers.

In the pattern tracing test, speed of movement of the stylus through the pattern was held constant. The measurement consisted of the number of contacts on the side of the pattern and their total duration in units of  $1/120$ ths of a second. In the reaction time test the subject, walking on the treadmill, turned off signal lights by tapping one of the two prescribed telegraph keys located 18 inches from the floor of the treadmill. A gross body movement is involved in bending over and striking these keys. The time score in  $\frac{1}{2}$  of a second is the average of 50 reactions. The ball-pipe test has been described elsewhere (8). Tapping was done with a stylus alternating between two plates which were separated by a small barrier to prevent artifacts due to muscle tremor. The score represents the number of taps made during the last ten seconds of a 30-second period.

**RESULTS.** The essential metabolic data are given in tables 2 and 3. It will be noted that during the second and third days on the fifth fast the men worked with a significantly higher blood sugar (see table 6). This was accompanied by a lower blood acetone on the evening of the second day and morning of the third day. The urine acetone on the second day of the fifth fast was lower and the urinary nitrogen slightly higher than on the first fast. Table 4 gives the work pulse and ventilation rates. In the fifth fast the men showed a slight improvement in pulse rate during the first day and a definite improvement in ventilation rate during the second and third days. The blood lactates after anerobic work showed no change between the two fasts. Blood ketone levels decreased 2.3 mgm. per cent after the 10-minute run on the morning of the second day and rose an average of 3.8 mgm. per cent after an hour's rest. There was no difference in these changes after 5 fasting periods.

The glucose tolerance test at the end of the fasting period showed a slightly



increased ability of the men to regulate the blood sugar level since the average two-hour blood sugar was 15 mgm. per cent lower on the fifth fast as compared to the first fast. Three of the 4 men showed blood sugars at this time which were 13 to 28 mgm. per cent less on the fifth than on the first fast.

TABLE 1

Schedule of 2½ day starvation experiments. Only those periods are listed in which work was performed or observations taken. "Work" refers to walking on a motor-driven treadmill at 3.5 m.p.h. and 10 per cent grade; "anerobic work" refers to a 75 second run at 9 m.p.h. and 8.6 per cent grade; "heavy work" refers to running at 6 m.p.h. and 5 per cent grade.

DAY	TIME	ACTIVITY	OBSERVATIONS
I	7:30- 8:00 a.m.	Rest	B.M.R. Blood for ketones, sugars and hemoglobin
	8:00- 9:00 a.m.	Work	R.Q. blood sugar and hemoglobin
	9:15- 9:30 a.m.	Work	Psychomotor battery
	10:00-11:00 a.m.	Work	R.Q. blood sugar and pulse
	12:00- 1:00 p.m.	Work	R.Q. blood sugar and pulse
	2:00- 3:00 p.m.	Work	R.Q. blood sugar and pulse
	3:00- 3:15 p.m.	Rest	Blood for ketones and lactate
	3:17 p.m.	Anerobic work	Blood lactates at 12 and 30 min. recovery
	4:30- 4:45 p.m.	Work	Psychomotor battery
II	9:15- 9:45 a.m.	Rest	B.M.R. Blood for ketones, sugars and hemoglobin
	9:50-10:00 a.m.	Heavy work	Blood ketone at end of work
	10:00-11:00 a.m.	Rest	Blood ketone one hour after work
	11:00-12:00 p.m.	Work	R.Q. blood sugar and pulse
	12:15-12:30 p.m.	Work	Psychomotor battery
	1:00- 2:00 p.m.	Work	R.Q. blood sugar and pulse
	3:00- 4:00 p.m.	Work	R.Q. blood sugar and pulse
	4:00- 4:30 p.m.	Rest	Blood for ketone and sugar
	4:30- 4:45 p.m.	Work	Psychomotor battery
III	8:30- 9:00 a.m.	Rest	B.M.R. Blood for ketones, sugars and hemoglobin
	9:00-10:00 a.m.	Work	R.Q. blood sugar and pulse
	10:15-10:30 a.m.	Work	Psychomotor battery
	10:30-10:45 a.m.	Rest	Blood for lactate
	10:45 a.m.	Anerobic work	Recovery blood lactate at 12 and 30 min.
	12:15		Glucose tolerance Blood sugars at 1 and 2 hours

Table 5 presents the results of the psychomotor tests. The back lift and hand dynamometer results are not tabulated since no difference was observed between the two fasts. These measurements of simple strength are remarkably resistant to change in acute starvation. Statistically significant improvements, as compared with the first fast, occurred in the fifth fast in reaction time and pattern tracing. The statistical analysis is presented in table 6. The other two tests showed a trend toward improvement.

INDIVIDUAL DIFFERENCES AND DISCUSSION. Individual differences in response during starvation occurred both during a given fast and between the two fasting conditions presented here. The variability of response to a single fast will be

TABLE 2

The comparison of the average results on the R.Q., blood sugar during work and blood acetone during rest obtained on four men doing hard work during the first and last of five successive fasts.

VARIABLE	NO. OF FAST	DAYS OF FASTING							
		1				2		3	
		Work Period*							
		1	2	3	4	1	2	3	1
R.Q. in work	1	0.86	0.83	0.82	0.79	0.75	0.76	0.75	0.74
	5	0.82	0.80	0.79	0.80	0.73	0.74	0.74	0.73
	Δ	-0.04	-0.03	-0.03	+0.1	-0.02	-0.02	-0.01	-0.01
Blood sugar in work	1	75	78	75	71	49	44	43	56
	5	70.0	76.0	77	75	63	56	61	64
	Δ	-5	-2	+2	+4	+14	+12	+18	+8
Blood acetone in rest	1				1.0	14.9		19.2	20.9
	5				1.8	14.8		16.0	15.8
	Δ				+0.8	-0.1		-3.2	-5.0

\* Work periods referred to here are only the one hour periods and do not include the psychomotor periods (see protocol).

TABLE 3

Comparison of 24 hour urine acetone and urine nitrogen of men doing hard work during the first and fifth fasts of five successive fasts.

VARIABLE	NO. OF FAST	DAYS OF FASTING	
		1	2
Urine acetone	1	0.788	4.788
	5	0.947	2.894
	Δ	+0.159	-1.894
Urine nitrogen	1	7.50†	10.98*
	5	8.64†	11.82*
	Δ	+1.14	+0.84

\* Two men.

† Three men.

presented elsewhere. The individual changes which occurred in the metabolic variables between the first and fifth fast are presented in table 7. The marked improvement after successive fasts by *Ja* and *No* in the ability to control blood sugar during work on the second day of fasting was accompanied by a parallel

TABLE 4

Comparison of work pulse and ventilation rates of 4 men doing hard work during the first and fifth fasts of five successive fasts. The men were working on a motor-driven treadmill at 3.5 m.p.h. and 10 per cent grade.

NO. OF FAST	DAY OF FASTING							
	1				2		3	
	Work Period*							
	1	2	3	4	1	2	3	1
Pulse rate beats/min.								
1	139	148	153	158	168	169	170	172
5	137	142	145	147	166	168	170	168
Δ	-2	-6	-8	-11	-2	-1	0	-4
Ventilation liters/min.								
1	33.5	33.5	33.8	34.3	37.5	37.9	38.2	39.8
5	31.3	31.6	31.3	31.1	32.9	34.2	33.2	32.5
Δ	2.2	1.9	2.5	3.2	4.6	3.7	5.0	7.3

\* Work periods referred to here are only the one-hour periods and do not include the psychomotor periods (see protocol).

TABLE 5

The results of the psychomotor tests of speed and co-ordination. The numbers listed in the columns under days of fasting represent the difference between the "control score" and the score at the specified time. The negative signs indicate deterioration in performance. High control scores on the ball-pipe test and tapping indicate good performance but poor performance in pattern tracing and reaction time.

VARIABLE	TEST	NO. OF FAST	AVERAGE CONTROL SCORE*	DAYS OF FASTING				
				1		2		3
				a.m.	p.m.	a.m.	p.m.	a.m.
Eye-hand co-ordination and steadiness	Pattern tracing:	1	38.3	-3.5	-6.8	-11.8	-25.8	-25.8
		5	22.0	-3.4	+1.0	-10.0	-17.0	-14.0
		Δ	+0.1	+7.8	+1.8	+8.8	+11.8	
	1) No. of contacts	1	198.8	-15.0	-33.3	-53.3	-180.0	-183.5
		5	94.1	-7.8	-3.8	-44.8	-88.8	-82.0
		Δ	+7.2	+29.5	+8.5	+91.2	+10.15	
2) Length of contacts	1	198.8	-15.0	-33.3	-53.3	-180.0	-183.5	
	5	94.1	-7.8	-3.8	-44.8	-88.8	-82.0	
	Δ	+7.2	+29.5	+8.5	+91.2	+10.15		
Arm-hand speed and co-ordination	Ball-pipe test	1	64.8	0	-2.0	-7.8	-12.5	-14.5
		5	72.2	+0.3	+1.8	-9.0	-2.8	+0.5
		Δ	+0.3	+3.8	-1.2	+9.7	+15.0	
Hand speed	Tapping	1	57.3	-5.0	+3.8	-0.3	-1.5	-7.8
		5	60.3	+3.0	+3.3	-2.3	0	-2.0
		Δ	+8.0	-0.5	-2.0	+1.5	+5.8	
Gross body speed	Reaction time	1	43.8	-3.3	-3.3	-8.3	-14.0	-19.2
		5	51.8	+2.9	+2.3	-7.5	-11.2	-13.5
		Δ	+6.2	+5.6	+0.8	+2.8	+5.7	

\* The "average control score" represents the average of morning and afternoon tests given 3 to 5 days before and 7 to 10 days after each fast.

improvement in ability to maintain blood ketones at a lower level. The nitrogen excretion data are incomplete but those available indicate that gluconeogenesis

TABLE 6

Statistical analysis of psychomotor and blood sugar data; all comparisons are between the first and fifth fasts at the specified time. The Fischer *t*-test for paired variates was used with 3 degrees of freedom.

VARIABLE COMPARED	TIME	t-VALUE	t-VALUE AT			
			1%	2%	5%	10%
Pattern tracing	p.m. 1st day	2.60				
No. of contacts	a.m. 3rd day	4.91	5.84	4.54	3.18	2.35
Pattern tracing	p.m. 1st day	3.42		4.54	3.18	
Length of contact	a.m. 3rd day	2.93				2.35
Ball-pipe	p.m. 1st day	0.06				
	a.m. 3rd day	1.63				
Tapping	p.m. 1st day	0.12				
	a.m. 3rd day	1.83				
Reaction time	p.m. 1st day	4.59	5.84	4.54		
	a.m. 3rd day	0.42				
Blood sugar	1st one-hour work period	7.15	5.84	4.54		
	2nd day					
	2nd one-hour work period	4.81	5.84	4.54		
	2nd day					

TABLE 7

The individual "improvements" in blood sugar, blood acetone, urine acetone and urine N which occurred between the first and fifth fasts. The sign indicates the absolute algebraic difference between the fifth and first fasts ( $V - I$ ). Blood variables are expressed as milligrams per cent and urine variables as grams excreted over indicated time.

VARIABLE	BLOOD SUGAR			BLOOD ACETONE	URINE ACETONE	URINE NITROGEN	
Day	2			3	1st two days	1	2
Time	Work Period No.			a.m.	48 hours	24 hours	24 hours
	1	2	3				
Ja	+18	+19	+24	-11	-7.5	+2.80	
No	+14	+20	+13	-7	-0.120	+0.61	+2.35
Se	+16	+7	+15	-2	-0.716	0.0	
Jo	+9	+5	+9	0	-0.947		-0.65

only played an important rôle in two men. The data of *Ja* and *No* are in general agreement with the observations of MacKay *et al.* (9) on the rôle of gluconeogenesis from protein in the control of ketosis and maintenance of blood sugar in fasting

rats. It would appear from the data of *Jo* that substantial gains in blood sugar regulation and in reduction of ketone production as measured by urinary excretion can be made without a parallel increase in nitrogen excretion. A similar situation was found by Folin and Dennis (1) who observed that both urinary ketones and nitrogen excretion diminished in two obese subjects on repeated fasting. The experiments reported here extend to non-obese persons this picture of a decrease in urinary ketones on successive fasts with the modification that this is not necessarily accompanied by a decreased N excretion.

Since it is generally believed that the nervous system burns only carbohydrate it might be expected that the improvements in psychomotor performance would parallel the increases in blood sugar. This did not occur. *Ja* made the largest relative gains and improved his performance on all five tests. *No* made the least improvement and actually showed some loss of ability in reaction time. *Jo* showed improvement on pipe and tapping while *Se* made improvements in tapping and pattern tracing. Both these men improved their reaction time on the evening of the first day. However, it should be kept in mind that this comparison is not too exact since blood sugar was not determined during the actual performance of the psychomotor tasks.

It is probable that the state of physical training plays an important rôle in the ability of men to perform hard work during fasting. It is felt that the 4 subjects were in the same state of physical fitness at the beginning of both the first and fifth fasts. The average pulse rates (walking) on the morning of the first day differed in the two fasts by only 2 beats per minute while the 12-minute lactate after a fixed anerobic work (90 sec. at 9 m.p.h. and 8.6 per cent grade), performed within a week of fasting, was 61.9 mgm. per cent in June and 64.7 mgm. per cent in October. The psychomotor performance was, in general, somewhat superior on the fifth fast as compared to the first. Sufficient data are not available to determine precisely the effect of this initial difference on deterioration under stress. However, the indications are that this difference was of little consequence.

Earlier observations on the effects of exercise on the blood ketone levels (10, 11, 12) have been confirmed. However, it appears that the measurement of blood ketones under these conditions yields no information on the ability of men to perform physical or psychomotor tasks.

The data presented here demonstrate that repeated exposure to the fasting state results in a more effective adaptation to fasting. The mechanism remains obscure. Gluconeogenesis from protein does not account satisfactorily for the increased ability to maintain blood sugar since it occurred definitely in only two men. It is possible that an increase in the amount of fat metabolized played a rôle in the conservation of carbohydrate but more extensive data would be necessary to establish this difference. The time period over which this effect will persist must be elucidated by further research. Our data cannot be interpreted to give information as to the number of fasts necessary to produce a significant effect. However the data of Folin and Dennis (1) indicate a trend which begins on the second fast.

A somewhat similar situation exists when men are exposed to hot environments. A stay in a hot environment for a few days reduces the stress of a second exposure and this effect lasts up to a month (13, 14, 15). The experience of the Everest Committee and other mountain groups is somewhat similar. Their records show that experienced high altitude mountaineers resist the stresses of high altitude climbing better than do the beginners. It appears then that there is a group of stresses which when applied at intervals to man induce subtle adaptive changes which make the organism more fit to withstand the stress on subsequent occasions.

#### SUMMARY AND CONCLUSIONS

1. Metabolic, physiological and psychomotor measurements have been carried out on 4 men who performed hard work under rigidly controlled conditions during 5 successive  $2\frac{1}{2}$  day fasts. The total caloric deficit was of the order of 10,000 calories. The successive fasts were separated by 5 to 6 week intervals. Results on the first and fifth fasts have been reported.

2. During the second and third days of fasting all men were able to maintain the blood sugar in work at a significantly higher level in the fifth as compared to the first fast.

3. Motor speed and co-ordination deteriorated less during the fifth fast. Reaction time and pattern tracing showed a statistically significant improvement while two other psychomotor tests showed trends in the same direction.

4. The relationships between blood sugar, blood ketone and urinary nitrogen have been discussed.

*Acknowledgment.* It is a pleasure to acknowledge the able and devoted assistance of the Staff of the Laboratory of Physiological Hygiene, without which this work could not have been accomplished. The subjects were on detached service in Civilian Public Service Camp No. 115. One of them, Walter Carlson, gave much help with the gas analysis.

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## THE EFFECT OF INSULIN ON PHOSPHORUS TURNOVER IN MUSCLE<sup>1, 2</sup>

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The mechanisms by which insulin produces its effects on the metabolism of carbohydrates are little better understood at the present time than they were when these effects were first described by Best and his co-workers (1). In the intervening years, the extensive study of enzyme systems has demonstrated the intimate connection between phosphorus compounds in tissues and the intermediary metabolism of carbohydrate. The use of radioactive phosphorus ( $P^{32}$ ) as a tracer in the intact animal has clarified to a considerable degree the exact function of these phosphorus compounds in the metabolism of striated muscle (6, 7, 8). The work reported below represents an extension of this tracer technique to the study of the mechanism of insulin action, and the results throw some light on the details of the part played by this hormone in the deposition of glycogen and oxidation of carbohydrate in muscle.

Since previous work (6, 7) has shown that the turnover rates of the several P compounds in muscle can be readily modified by a variety of conditions well within the physiological range, the experiments were so designed as to permit the study of several of these variables. The turnover rate is different in fasting and in the post-absorptive state; muscular activity, even when intense and relatively prolonged, does not influence the turnover rate, but there is a markedly increased turnover in recovery from such activity; the administration of glucose reduces the turnover rate in the post-absorptive state, but not in fasting. The experiments with insulin were therefore carried out on both fasting and post-absorptive animals, in resting muscles and in those recovering from prolonged activity. To avoid the complications that would be introduced by hypoglycemia, glucose was given to all animals. The dose of insulin used, 5 units per kgm., is probably greater than that needed to evoke minimal effects, but it was felt to be more desirable to look for significant differences than for those whose evaluation would depend on statistical analysis of the results.

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<sup>2</sup>A preliminary report on this appeared in *Science* 98: 388, 1943.

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As in previous studies, the experiments were carried out on cats. Those animals which had last been fed late in the afternoon previous to the experiment were regarded as being in the post-absorptive state; those last fed on the second day prior to the experiment were considered to be in the fasting state. After the induction of anesthesia with pentobarbital, the  $P^{32}$ , in the form of  $Na_2HPO_4$ , was injected subcutaneously. This was followed in half an hour by the intraperitoneal injection of glucose, 1.5 grams per kgm., in 5 per cent solution. When insulin was given, it was injected subcutaneously 30 minutes after the glucose. One hour and 45 minutes after the injection of the tracer phosphate, one gastrocnemius muscle was stimulated at the rate of one twitch per second against moderate initial tension, for a period of 15 minutes. The tension was then removed, and the muscle allowed to recover in the relaxed state for a period of 2 hours. At the end of this time, i.e., 4 hours after the injection of the  $P^{32}$ , a sample of arterial blood was collected and heparinized, and the two gastrocnemius muscles then frozen in the usual way. Trichloroacetic acid filtrates of muscle and plasma were prepared, and from these the P originally present as muscle phosphocreatine (PC), the two labile phosphate groups of adenosine triphosphate (ATP), fructose-6-phosphate (FP), glucose-6-phosphate (GP), and plasma inorganic phosphate, was separated and prepared for the determination of radioactivity by the methods previously described (6, 8). Measurements of radioactivity were made by a Geiger-Müller counter.

The results of the experiments, which are given in table 1, indicate that in resting muscle insulin brings about an increase in the turnover rates of all four P compounds, both in the fasting and post-absorptive states; the increased turnover in recovery from activity which occurs normally, fails to appear under the influence of this dose of insulin; under certain limited conditions insulin seems to increase the amount of GP adsorbed on the cell membrane, and, by inference from this, to increase the rate of entrance of glucose into the cell interior and presumably the rate of deposition of muscle glycogen. Also, the mechanism by which the administration of glucose to post-absorptive animals results in a decreased P turnover in the muscles of post-absorptive animals, continues to operate during insulin action. Consequently, the  $P^{32}$  levels reached in post-absorptive animals given insulin and glucose are below those reached in fasted animals similarly treated.

To facilitate the comparison of results on the various groups, all the measurements of radioactivity have been expressed in terms of counts per minute registered on the Geiger-Müller counter per mgm. of P, calculated to the basis of one million counts per minute of tracer material injected per kgm. body weight. Suitable corrections for radioactive decay have naturally been made.

In resting muscle the turnover rates of PC and ATP are more than doubled under the influence of the injected insulin, in both fasted and post-absorptive animals. In view of the abundant evidence from the study of enzyme systems that the oxidation of glucose is associated with phosphorylations, this increased P turnover may well represent the acceleration of glucose oxidation that insulin is known to bring about in muscle (1). To make such a correlation, it is necessary

TABLE 1

*Effect of insulin on phosphorus turnover to muscles of cats given glucose*

Values are expressed as counts per minute per mgm. P, calculated to the basis of one million counts per minute injected per kgm. body weight.

RESTING				STIMULATED AND RECOVERING				PLASMA INORG. P	
Phospho- creatine	Adenosine triphos- phate	Fructose- 6-phos- phate	Glucose- 6-phos- phate	Phospho- creatine	Adenosine triphos- phate	Fructose- 6-phos- phate	Glucose- 6-phos- phate	Relative radio- activity	Mgm. Per cent
A. Fasting animals									
210	106		665	191	133		830	14200	4.9
87	63		402	106	95		342	7150	5.5
88	84	50	610	108	96	59	855	8650	5.3
52	72	103	385	70	76	50	396	7000	5.0
R 67	64		495					8000	8.0
L 76	70		434						
R 158	83	72	590					8550	7.4
L 88	73	38	525						
Av.: 103	77	66	514	119	100	55	606	8925	6.0
B. Fasting animals given insulin									
425	176	238	380	320	241	121	425	4950	3.8
205	133	80	374	187	146	175	508	6350	4.9
257	198	114	555	276	196	235	980	4680	6.0
380	248	119	620	299	238	180	1020	8800	2.6
Av.: 316	189	138	482	271	205	203	733	6195	4.3
C. Post absorptive animals									
89	87	67	70	90	101	71	75	7350	6.1
80	87	68	60	124	108	78	70	6570	5.9
67	58	55	38	89	85	73	74	6550	8.3
131	76	51	37	147	118	41	59	6250	6.2
R 46	51	32	47					6100	10.1
L 39	56	17	30						
R 105	99		38					5900	8.9
L 75	92	50	94						
Av.: 75	76	49	52	113	103	66	70	6453	7.6
D. Post absorptive animals given insulin									
122	117	75	102	167	151	120	126	6150	6.6
154	116	97	87	155	145	143	134	5100	5.2
172	148	112	110	200	150	108	107	5800	3.2
298	235	160	117	189	178	107	98	7800	2.5
Av.: 187	154	111	104	203	156	120	116	6142	4.4
E. Average values for post-absorptive animals not given glucose. (Reprinted from This Journal, 142: 621, 1944)									
Av.: 154	143	81	56	234	217	131	73		

to postulate that the increased turnover denoted by the higher  $P^{32}$  content reached in the same time interval, is necessarily accompanied by an accelerated rate of passage of phosphate groups across the cell membrane. This migration of phosphate groups has been shown to take place primarily by the formation of PC and ATP on the cell membrane, with penetration of the newly forming molecules through the membrane (8). Since the total P present in any one form in the cell remains constant, the acceleration of formation of these compounds at the membrane must be accompanied by an equal acceleration of their rate of breakdown at the membrane. In the absence of such an accelerated rate of passage across the membrane, the relative  $P^{32}$  contents would give no indication of change in intracellular turnover rate.

Since the effect of insulin is to increase the turnover rates of these compounds, the lower turnover rates in fasting may well be due to diminished insulin secretion in this state, as a consequence of the lowered insulin content of the pancreas in the fasting animals (2). The effect of glucose administration in reducing the turnover rate in the post-absorptive state must, of course, be ascribed to some other agency than insulin.

In the muscles undergoing recovery from prolonged activity, the effect of insulin is apparently to diminish or suppress the increased turnover of PC and ATP which is seen in the absence of an external supply of this hormone. Apparently the effect is one of actual inhibition, since the levels of  $P^{32}$  reached are below those found in the recovering muscles of post-absorptive animals without the administration of either glucose or insulin, and are not significantly greater than in the companion resting muscles.

As has been pointed out previously, the radioactivity measurements on the FP fraction are significant only when taken in connection with those for GP (6). In the post-absorptive animal, the relative figures do give an idea of the metabolic turnover of this substance, whereas those on the fasting animal reflect to a more marked degree the admixture of material derived from the GP in the incomplete separation that is possible. The data obtained serve to emphasize the point that the metabolic pathways of the two phosphorylated hexoses are unrelated in this species, for there is no relation between the apparent turnover rates of the two substances. This is not general for all species, for the two compounds have been found to be interconvertible in the muscles of the frog (6).

In the post-absorptive state, the GP shows about the same increase in turnover rate under insulin as do the PC and ATP. Under these conditions, the  $P^{32}$  content of the GP, in relation to those of the PC and ATP, does represent the general metabolic turnover rate. In fasting animals this is not the case. Instead, it serves as an index of the extent to which formation and adsorption of GP has taken place on the cell membrane. This reaction has been shown (6) to be the first step in the absorption of glucose by the muscle cell. The absence of an increased  $P^{32}$  level in the GP of the recovering muscles of the post-absorptive animals under insulin, indicates a very slow rate of resynthesis of muscle glycogen under these conditions. This slow rate of deposition of muscle glycogen in recovery has been reported in numerous investigations.

In the fasting animal, the high  $P^{32}$  content of the GP in the resting muscle is not raised by insulin. In the muscles recovering from activity, insulin seems to produce a significant increase; it therefore seems to increase the amount of GP on the cell membrane. Thus it is only under the special condition of recovery from prolonged activity in the muscles of a fasting animal that insulin seems to increase the rate of the first phase of glucose absorption by the cell. Depletion of the glycogen stores by activity does not necessarily of itself act as an adequate stimulus to an increased rate of glucose absorption under the influence of insulin; only when that depletion takes place in a fasted animal does insulin evoke an increased rate of formation of GP on the membrane, the initial step in glucose absorption.

The maximum  $P^{32}$  content in the GP in any of these experiments, roughly one-tenth that of the plasma inorganic P, represents the presence on the cell membrane of roughly 1 to 2 mgm. per cent of P as GP, since the GP content of the muscle is less than 10 mgm. per cent, as P. Such a quantity could scarcely be detected by ordinary chemical means. This figure does not give any clue to the rate of passage of glucose into the cell interior; however, it may be assumed that the rate of passage would be much higher when the amount of adsorbed GP is high than when it is vanishingly small, as must be the case in the post-absorptive animals.

The demonstration that GP serves as the mechanism by which glucose enters the muscle cell interior, and that it does not enter into exchange reactions with the other intracellular organic P compounds, makes possible a tentative evaluation of the rôle of glucose-1-phosphate, the Cori ester (3), in the metabolism of the intact cell. It may well be that the glucose molecule, after entrance into the cell, is phosphorylated in the 1-position by some enzyme not yet identified, and that under the influence of phosphorylase the Cori ester is converted to glycogen and inorganic phosphate. In this way, the formation of glycogen could take place by phosphorolysis without a phosphate group on the 6-position of glucose being involved.

In the present experiments, the GP content of the muscles, as distinguished from the  $P^{32}$  content of the GP, was not affected by insulin. This is in contrast to the observations of Kaplan and Greenberg (4) on rabbits given insulin and  $P^{32}$ . They report a doubling of both the content of barium-soluble P and of the  $P^{32}$  content of this P, under insulin. The fractionation procedure they used would result in a barium-soluble fraction consisting largely, if not entirely, of hexosemonophosphate. Examination of their data shows that the increase in the amount of P in the barium-soluble fraction was derived from the PC, and that the increased  $P^{32}$  could have come only from plasma inorganic phosphate. These data indicate that insulin in the rabbit has a much greater effect on the formation of hexosemonophosphate on the cell membrane than in the cat. Since Kaplan and Greenberg did not fractionate the hexosemonophosphate into FP and GP, their data do not throw any light on the question of species differences in the behavior of these two compounds.

As has been reported repeatedly, insulin was found to lower the plasma inorganic phosphate level. Only in the fasted animals did it bring about a decrease

in the  $P^{32}$  content of this material. This combination of lowered total plasma P with increased  $P^{32}$  levels in the muscle compounds, may indicate a net migration of P into the muscle cell interior under the influence of insulin, such as that found in the liver by Nelson *et al.* (5). However, any such migration would have to be vanishingly small in relation to the amount of intracellular organic phosphate present in muscle.

The author is indebted to Prof. J. M. Cork of the Department of Physics, University of Michigan, and to Dr. J. G. Hamilton of the Radiation Laboratory, University of California, for the generous supplies of  $P^{32}$  for these experiments.

#### SUMMARY AND CONCLUSIONS

1. The effect of insulin on phosphate turnover in striated muscle has been studied in cats by means of radioactive phosphorus ( $P^{32}$ ).

2. In both fasting and post-absorptive animals given glucose, insulin increases the turnover rates of phosphocreatine and the two labile phosphate groups of adenosine triphosphate, in resting muscle. It also increased the turnover rate of glucose-6-phosphate in resting muscles of post-absorptive animals.

3. The increase in turnover of P which normally takes place in the post-absorptive state, in recovery from prolonged activity, is not seen when insulin is administered.

4. Insulin does not seem to modify the extent of formation of glucose-6-phosphate on the muscle cell membrane in resting muscles of either fasting or post-absorptive animals.

5. Insulin seems to increase the formation of glucose-6-phosphate on the cell membrane during recovery from muscular activity, in fasted, but not in post-absorptive animals.

6. Insulin does not counteract the effect of glucose in reducing P turnover in the post-absorptive state.

7. The evidence indicates that insulin accelerates the rate of glucose oxidation within the muscle cell, but that other factors are of greater importance in determining the rate of passage of glucose across the cell membrane.

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# GASTRIC CARDIOSPASM IN THE DOG

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In a previous paper (1) we have reported on the ineffectiveness of atropine and other spasmolytic agents in abolishing gastric cardiospasm of the dog following bilateral vagotomy in the neck. In this, the cardia seems to differ from other smooth muscle of the gastro-intestinal tract. Contradictory statements are found in the literature (2-8) as to the mechanism of this type of cardiospasm. This lack of uniformity of opinion on the response of the cardia to section and stimulation of nerves led to this investigation.

**METHOD.** In 53 barbitalized dogs cardiac function was observed essentially as by Langley (2), that is, measuring the resistance to inflowing water. A glass tube was tied into the esophagus at the neck and connected to an upright glass tube serving as a manometer. A constant flow of fluid from a Mariotte bottle into the manometer tube, the upper end of which was connected to a volume recorder, permitted continuous recording. In most experiments the glass tube used was short in order to observe the responses of both the esophagus and the cardia. In a few experiments, to exclude recording of esophageal activity, a long tube reaching down to the diaphragm was used. In this case the esophagus was fastened to the lower end of the tube by means of a wide rubber band to prevent leakage of fluid into the lumen of the esophagus. The outflow of the water was rendered possible by insertion of a very wide glass tube into the stomach wall. To exclude spontaneous respiratory movements both phrenic nerves were cut in the neck and a bilateral pneumothorax was done, and positive pressure artificial respiration was applied. A variable frequency electron tube device was used for nerve stimulation. When nerves close to the diaphragm were stimulated, the electrode was put into a rubber condom to avoid leakage of current.

**EXPERIMENTS.** If the vagi are cut about 3 cm. above the diaphragm, there is no change in the cardia but their subsequent section in the neck is followed by spasm (fig. 1, A, B). In 9 out of 42 dogs, on which bilateral vagotomy in the neck was performed, the cardia remained unchanged, presumably due to the depth of anesthesia. If the peripheral right vagus is now stimulated in the neck, the resistance to flow rises immediately and remains high during the stimulation, while after stimulation a fall in resistance occurs (fig. 1 C). Stimulation of the peripheral end of the vagus, cut just above the diaphragm, causes a rise in resistance (fig. 1 D).

If the peripheral end of the right or left vagus is stimulated, after section of both nerves in the neck only, with following spasm of the cardia, three responses are seen. Comparatively weak stimuli give the immediate rise during stimulation and the fall afterwards, while a stronger stimulus adds the third effect, the delayed rise (fig. 2, A, B). After curare the immediate rise and the subsequent

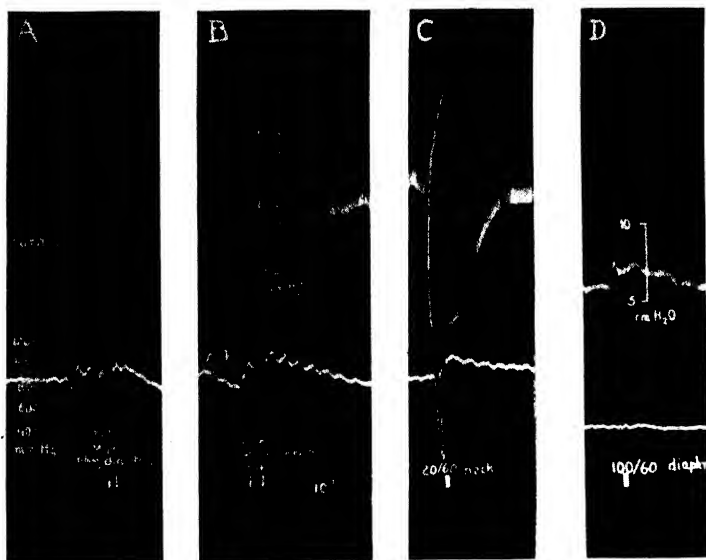


Fig. 1. Dog 17.5 kgm., barbitol anesthesia. Short tube in esophagus. From the top down, cardia, carotid arterial pressure, signal of nerve stimulation, time in 10 seconds.

A. Both vagi cut at level of diaphragm. B. Both vagi cut in the neck. C. Stimulation of right vagus in the neck, frequency 60 per second. D. Different experiment. Dog 10.2 kgm., barbitol anesthesia, stimulation of right vagus at level of diaphragm, frequency 60 per second, intensity much greater than at C.

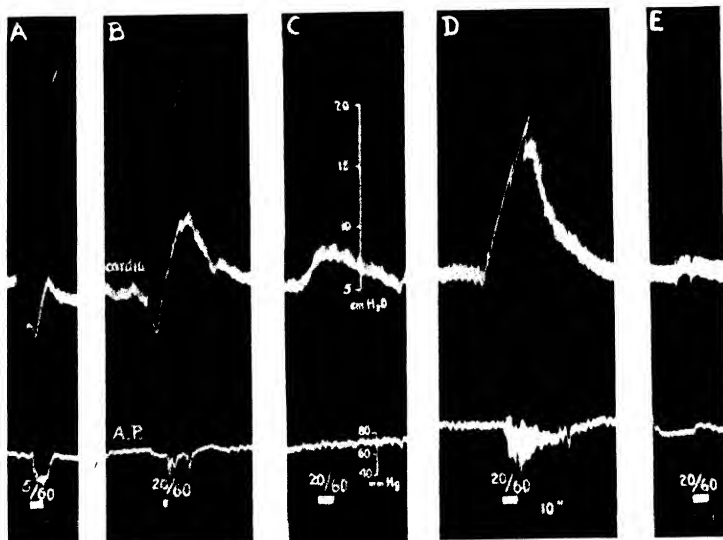


Fig. 2. Dog 18 kgm., barbitol anesthesia. Short tube in esophagus. From the top down, cardia, carotid arterial pressure, signal of nerve stimulation, time in 10 seconds. After section of both vagi in the neck.

A. Stimulation of right vagus in the neck, frequency 60 per second. B. Stimulation of right vagus in the neck, frequency 60 per second, greater intensity than at A. C. Same as B after the injection of curare. D. Same as B after the intravenous injection of 0.25 mgm./kgm. physostigmine. E. Same as B after the intravenous injection of 1.0 mgm./kgm. atropine.

fall are reduced (fig. 2 C). Progressive slow curarization with erythroidine abolishes both effects concomitantly (fig. 4). The delayed rise is augmented by eserine and abolished by atropine, although atropine is not very effective in abolishing spasm of the cardia (fig. 2, D, E). Clearly, the immediate rise is from contraction of the striated muscle of the esophagus, since it does not appear on the record if a long tube is used (fig. 3), the late rise is from contraction of the cardia.

Stimulation of either vagus in the neck is without any effect on the tone of the cardia, if the esophagus is sectioned about 2 cm. above the diaphragm, while care is being taken to avoid injury to the vagus nerves (fig. 3).



Fig. 3

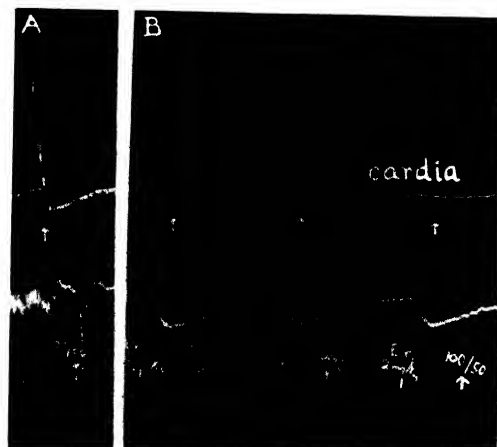


Fig. 4

Fig. 3. Dog 13.6 kgm., barbital anesthesia. Long tube in esophagus. From the top down, cardia, carotid arterial pressure, signal of nerve stimulation, time in minutes.

A. Stimulation of right vagus in the neck, frequency 50 per second. B. Same as A after section of the esophagus at the diaphragm.

Fig. 4. Dog 15 kgm., barbital anesthesia. Short tube in esophagus. From the top down, cardia, carotid arterial pressure, signal of nerve stimulation and injection, time in minutes. At arrows stimulation of right vagus in the neck, at signals injections of 2 mgm./kgm. erythroidine hydrochloride. Between A and B two injections of 2.0 mgm./kgm. of erythroidine hydrochloride.

Stimulation of the left splanchnic nerve, during spasm of the cardia, produces a slowly developing rise in resistance, which is augmented by cocaine. Stimulation of the lower thoracic sympathetic gives an immediate and a delayed rise in pressure. To gain insight into the mechanism of the effects of nerve stimulation in the light of the modern theory of humoral transmission of nerve impulses, we studied the effects of epinephrine and acetyl-choline on the cardia. Both cause contraction of the cardia on their injection into the left gastric artery by way of the central end of the splenic artery, with the common hepatic artery ligated. On intravenous injection, acetyl-choline has little effect in small doses, and the action of large doses is complicated by the release of adrenin. Epinephrine intravenously causes slight contraction of the cardia, but intense contraction after



bilateral vagotomy in the neck (figs. 5, A, B, and 6, A, B). It certainly does not constrict the open cardia and relax the closed cardia as has been claimed (4). Cocaine augments this action of epinephrine (fig. 5 C), and ergotamine, which in small doses gives a fall in resistance of the cardia (fig. 6 C), reverses the effect (fig. 6 D).

**DISCUSSION.** It appears that the vagus nerves supply the cardia both with motor and inhibitory fibers. The former are cholinergic in nature since the motor effect following vagal stimulation is augmented by eserine and abolished

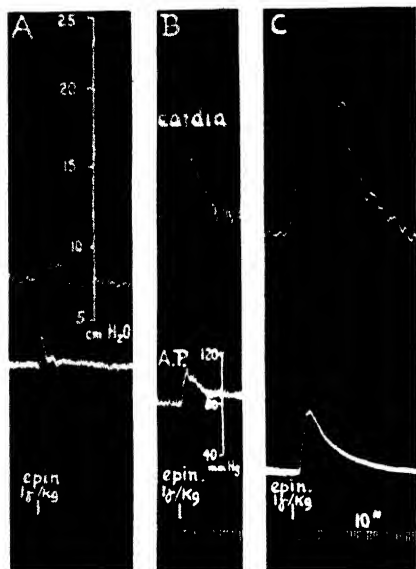


Fig. 5

Fig. 5. Dog 11.8 kgm., barbitol anesthesia. Short tube in esophagus. From the top down, cardia, carotid arterial pressure, signal of injection, time in 10 seconds.

A. One microgram per kgm. epinephrine intravenously. B. Same as A after section of both vagi in the neck. C. Same as B after the intravenous injection of 2.0 mgm./kgm. cocaine hydrochloride.

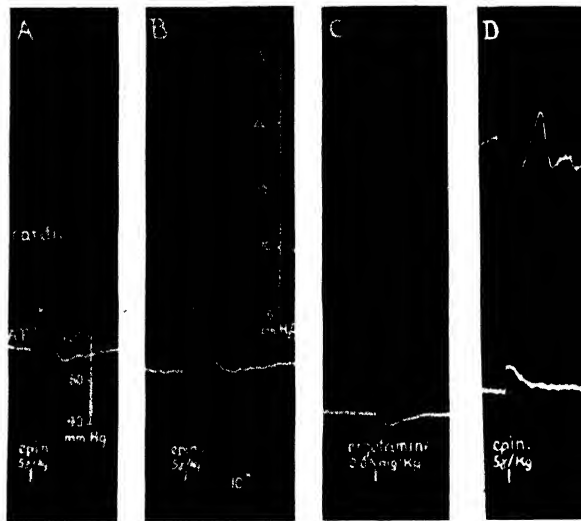


Fig. 6

Fig. 6. Dog 6.5 kgm., barbitol anesthesia. Short tube in esophagus. From the top down, cardia, carotid arterial pressure, signal of injection, time in 10 seconds.

A. Five micrograms per kgm. epinephrine intravenously. B. Same as A after section of both vagi in the neck. C. Three-hundredths mgm./kgm. ergotamine intravenously. D. Five micrograms per kgm. epinephrine intravenously.

by atropine. As epinephrine and acetyl-choline cause contraction of the cardia, neither cholinergic nor adrenergic mechanisms seem adequate to explain the inhibitory effect which occurs after vagal stimulation in the neck.

It is evident that the motor fibers supplying the cardia travel in the main trunks of the vagus nerve, since stimulation in the neck and at the level of the diaphragm have the same motor effect. However, the mechanism producing relaxation of the cardia is within the wall of the lower esophagus. The evidence for this is:

1. Section of both vagi at the diaphragm does not cause cardiospasm, but subsequent section in the neck does.

2. Stimulation in the neck after section at the diaphragm still causes inhibition of the cardia. Thus relaxation from vagal stimulation in the neck does not depend on the integrity of the vagi just before they reach the cardia.

3. Stimulation of the vagus at the diaphragm never produces inhibition of the cardia.

4. After the esophagus has been sectioned at the diaphragm (vagi intact), stimulation of the vagus in the neck produces no change in the tone of the cardia.

Two interpretations are possible as to the nature of this inhibitory mechanism. One possibility is that the vagus nerves carry true inhibitory fibers, which enter the wall of the lower esophagus before they reach the cardia. If this were the case, these fibers certainly would be preganglionic, since after curare, stimulation of the cervical vagus has no inhibitory effect.

The other possible explanation is that esophageal contraction produces by some intrinsic mechanism relaxation of the cardia as suggested by Burget and Zeller (7), and when esophageal activity is abolished by vagotomy, cardiospasm results. The fact that distention of the lower end of the esophagus (7), similar to vagal stimulation, produces relaxation of the cardia lends some support to the latter hypothesis, as does the effect of progressive, slow curarization.

Removal of this inhibitory mechanism by vagal section results in cardiospasm presumably due to predominance of the sympathetic system (6), inasmuch as the cardia is more sensitive to epinephrine under this condition. The relaxing effect of ergotamine affirms this belief of the predominance of the sympathetic, which is motor in its effect on the cardia.

There seems to be some relationship between experimental and clinical cardiospasm, since it was demonstrated that the latter condition is frequently associated with pathological changes in the vagus nerves and in ganglion cells of the lower esophagus and cardia (9, 10).

#### SUMMARY

The response of the cardia to section and stimulation of nerves and to the administration of autonomic drugs has been studied in 53 barbitalized dogs.

Stimulation of the cervical vagus causes relaxation of the cardia, followed by contraction.

The fibers producing contraction are cholinergic, they travel in the main trunks of the vagus nerve.

The inhibitory mechanism is within the wall of the lower esophagus.

Two possible hypotheses are discussed as to the nature of this inhibitory mechanism.

Interference with this inhibitory mechanism by bilateral vagotomy in the neck is followed by cardiospasm due to predominance of the sympathetic which is motor in its effect on the cardia.

Ergotamine causes relaxation of the cardia.

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## ADRENAL CORTEX AND WORK IN THE HEAT<sup>1</sup>

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This paper deals with the effects of adrenocortical extract on men working in the heat. In testing the effects of any drug, comparison has to be made with the influence of at least 3 separate factors known to be of the utmost importance in the regulation of heat balance during work. Acclimatization for work in the heat (Robinson, Turrell, Belding and Horvath, 1943) is characterized by progressive improvement in the performance of a fixed task, as displayed by improvement in body temperature, pulse rate and comfort and by decreased susceptibility to heat exhaustion. Administration of ample amounts of water during work (Pitts, Johnson and Consolazio, 1944) results in striking improvement in the body's maintenance of thermal equilibrium over a period of hours, and protects the subject against exhaustion. Inadequate intake of sodium chloride predisposes in a matter of days to heat exhaustion (Taylor, Henschel, Mickelsen and Keys, 1943), which is avoided by adequate daily supplies of salt. In order to be of practical utility, any drug would have to demonstrate beneficial effects over and above those of acclimatization, water and salt.

Extract of adrenal cortex was studied because of reports that its administration is beneficial in therapeutic hyperthermia. Edelmann, Mahanna, Lewis, Thatcher and Hartman (1943) ameliorated the nausea and fatigue and accelerated the recuperation of luetics by injecting adrenocortical extract during their course of weekly fever treatments. The extract's chemical effects in these patients were to sustain the level of sodium in the plasma, to reduce the sodium concentration in their sweat, and to raise the blood glucose. The hemoconcentration usually produced in them by the fever was unaffected.

In the present series of experiments fully acclimatized subjects were living on a constant diet adequate in salt, and it was possible to obtain a satisfactory comparison between the effects of the drug and of water. Complete studies were made of physical efficiency during work, of respiratory exchange, of chemical balance and of the chemistry of sweat.

**METHODS.** Because of limitations in the supply of cortical extract only 2 subjects (J. S. and R. W.) were used. They were healthy young men, in good general physical training and fully acclimatized, as judged by the criteria of Robinson, Turrell, Belding and Horvath (1943), for work in moist heat by 2 months of almost daily marching in the hot room. During the test period they

<sup>1</sup> This work was conducted under a contract, recommended by the Committee on Medical Research, between the office of Scientific Research and Development and the President and Fellows of Harvard College.

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followed a rigid daily schedule of 4 hours in the hot room as subjects, 4 hours in the hot room as technicians and the rest of the day and night in a temperate environment. Their daily diet was constant; it provided by analysis 9.9 grams of Cl, 7.5 grams of Na and 3.1 grams of K for J. S. and 7.4, 6.0 and 2.2 grams respectively for R. W., and was good by National Research Council standards. Complete account was made of all fluid and food consumption; of all excreta, including sweat; and of daily activity.

Their responses to the heat were measured during work in a room maintained at  $90^{\circ}\text{F.} \pm 0.5^{\circ}\text{F.}$  and relative humidity 80 per cent  $\pm 1$  per cent. After 20 minutes for "warming up" they marched daily 3 consecutive hours on a motor driven treadmill up a 4 per cent grade at 3.5 m.p.h., wind velocity 5 m.p.h., with 10 minutes' rest each hour. The following physiological observations were made: pulse rate by palpation every 15 minutes during marching; rectal temperature by clinical thermometers at the end of each hour; blood pressure by mercury sphygmomanometer and auscultation at the eighth minute in every rest period; rate of sweating by loss in body weight; respiratory exchange in the first and third hours by analysis of expired air collected in a Tissot gasometer; skin temperature on the right forearm in the first and third hours by means of a copper-constantan couple read galvanometrically.

Samples of blood were collected by venepuncture before and after each day's march. The following analyses were made: hemoglobin, hematocrit and serum protein by the specific gravity method of Phillips, Van Slyke and colleagues (1943); serum chloride by the method of Harvey (1910); serum non-protein nitrogen by Nesslerization (Daly, 1933); serum sodium by the method of Butler and Tuthill (1931); serum potassium by the method of Shohl and Bennett (1928).

Samples of sweat were collected in the first and third hours by means of obstetrical rubber gloves fitted at the elbow with rubber bands fitting snugly but not impeding the circulation, and drained through a spigot on the little finger. Estimations of chloride, non-protein nitrogen, sodium and potassium were performed by the methods listed above.

Samples of urine were collected continuously in 12 hour periods, covering night and day respectively, and the chloride, sodium and potassium were estimated by the above methods. In addition total nitrogen was estimated by the micro-Kjeldahl technique of Ma and Zuazaga (1942).

In testing the effects of the drug, separate days' experiments on each subject were carried out under the following conditions: *a*, no drinking water, no drug; *b*, no drinking water, but drug; *c*, water to drink every 15 minutes at a rate equal to  $\frac{2}{3}$  the rate of sweating,<sup>3</sup> no drug; *d*, water to drink, drug. Water consumption was not controlled during the part of the day not devoted to marching. The order of the experiments was arranged so that both subjects did not receive the same treatment on any given day and therefore acted as controls for one another as well as for themselves.

<sup>3</sup> This amount of water to drink maintains reasonably good hydration for periods up to 4 hours (see Pitts, Johnson and Consolazio, 1944) and is the maximum tolerated by most men without undue gastric distention with consequent mild distress.

The adrenal cortical extract was Dr. E. C. Kendall's Extract 777 of whole beef adrenal cortex, proved potent by its effect on adrenalectomized rats. The dosage was 45 ml. injected intravenously just before the start of the day's work, this large dose being employed so as to obtain unequivocal evidence of any possible effects.

**RESULTS AND CONCLUSIONS.** Examination of the data will be facilitated by making 4 sets of comparison in every group of measurements: *a*, the absolute levels in the first and third hours; *b*, the increment or decrement from the first to the third hour, as a measure of the subject's ability to maintain a steady state; *c*, absolute levels and changes in experiments without and with water to drink, as a measure of the effects of water; *d*, absolute levels and changes in experiments without and with adrenocortical extract as a measure of the effects of the drug.

TABLE 1

*Rectal temperature, pulse rate and sweating as affected by administration of water, adrenal cortical extract or both*

(For experimental conditions see text)

EXPERIMENTAL CONDITIONS	RECTAL TEMPERATURE			PULSE RATE			RATE OF SWEATING			TOTAL SWEAT IN 3 HOURS
	1st hr.	3rd hr.	Δ	1st. hr.	3rd. hr.	Δ	1st hr.	3rd hr.	Δ	
	°F.	°F.	°F.	beats/min.			l./hr.	l./hr.	l./hr.	
Subject R. W.										
No water.....	101.4	103.5	+2.1	150	182	+32	1.56	1.22	-0.34	4.36
No water, cortex...	101.5	103.5	+2.0	148	176	+28	1.55	1.30	-0.25	4.57
Water alone.....	100.8	101.2	+0.4	124	130	+6	1.35	1.40	+0.05	4.25
Water and cortex...	100.8	101.3	+0.5	134	162	+28	1.70	1.48	-0.22	5.06
Subject J. S.										
No water.....	100.7	102.4	+1.7	128	172	+44	1.39	1.35	-0.04	4.17
No water, cortex...	100.7	102.4	+1.7	128	168	+40	1.57	1.23	-0.34	4.22
Water alone.....	100.4	100.7	+0.3	126	142	+16	1.43	1.32	-0.11	4.25
Water and cortex...	100.5	100.6	+0.1	122	130	+8	1.29	1.10	-0.18	3.59

*a. Responses to heat.* Pulse rate, body temperature, rate of sweating and blood pressure are the most significant measures of the subject's ability to maintain satisfactory condition in the heat (table 1). The rectal temperature rose steadily when water was withheld, remained within comfortable limits when it was drunk, and was unaffected by adrenal cortex. The pulse rate behaved similarly, except in R. W. whose pulse rate was high when water was drunk and extract was injected. Rate of sweating and total loss of sweat showed no effects attributable either to water or to adrenal cortex. The decrease in the third hour is characteristic of prolonged work in the heat (Johnson, Pitts and Consolazio, 1944). Blood pressure showed no changes attributable to either water or adrenal cortex (table 2). The subjects never reported subjective impressions either favorable or unfavorable to the extract, and it apparently had no effect on their performance in the heat.

b. *Respiratory exchange.* Progressive dehydration is usually accompanied by progressive deterioration in the mechanical efficiency of performing a fixed task, so that the oxygen consumption rises. Table 2 shows the rise of pulmonary ventilation and oxygen consumption characteristic of dehydration, the beneficial effects of water, and no significant effect of adrenal cortex. In addition, it shows that administration of water decreased the excretion of carbon dioxide, especially in the third hour. This was also unaffected by adrenal cortex. These results are at variance with the finding of Missiuro, Dill and Edwards (1938) that adrenal cortex improved the mechanical efficiency of normal men in moderate exercise. However, their experimental conditions were so different from ours that no direct comparison of results is profitable.

TABLE 2

*Respiratory exchange and blood pressure as affected by administration of water, adrenal cortical extract or both*

(For experimental conditions see text)

EXPERIMENTAL CONDITIONS	PULMONARY VENTILATION			OXYGEN CONSUMPTION			CARBON DIOXIDE EXCRETION			BLOOD PRESSURE (SYSTOLIC/DIASTOLIC)		
	1st hr.	3rd hr.	Δ	1st hr.	3rd hr.	Δ	1st hr.	3rd hr.	Δ	1st hr.	3rd hr.	Δ
	ml./kgm./min.			ml./kgm./min.			ml./kgm./min.			mm. Hg		
Subject R. W.												
No water.....	431	524	+93	23.9	26.1	+2.2	18.9	20.6	+1.7	126/70	112/76	-14/+6
No water, cortex..	422	548	+126	23.4	25.8	+2.4	18.0	20.1	+2.1	118/80	120/78	+2/-2
Water alone.....	434	445	+11	23.8	24.4	+0.6	19.0	18.3	-0.7	120/60	120/76	0/+16
Water and cortex..	446	467	+21	24.2	24.9	+0.7	18.6	18.9	+0.3	126/80	128/86	+2/+6
Subject J. S.												
No water.....	408	415	+7	19.6	21.0	+1.4	16.9	16.4	-0.5	122/76	118/76	-4/0
No water, cortex..	386	396	+10	19.0	21.4	+2.4	16.9	16.5	-0.4	126/76	108/76	-18/0
Water alone.....	382	382	0	19.4	20.3	+0.9	16.1	15.4	-0.7	126/86	110/86	-16/0
Water and cortex..	391	375	-16	19.1	19.7	+0.6	16.8	15.5	-1.3	116/82	114/82	-2/0

c. *Changes in blood chemistry.* Inspection of table 3 shows that with progressive dehydration serum sodium and serum non-protein nitrogen increased. (Whole blood hemoglobin and hematocrit and serum protein are not listed because they showed changes of the same order of magnitude and in the same direction as the serum sodium.) Serum chloride and serum potassium showed no regular changes in dehydration. Administration of water tended to prevent almost completely the rise in sodium, to a lesser extent the rise in non-protein nitrogen, and was without effect on serum chloride and serum potassium. Administration of cortical extract was without consistent effect on any of the substances measured. The findings of Edelmann, Mahanna, Lewis, Thatcher and Hartman (1943) in therapeutic hyperthermia were a drop in serum sodium, irregular behavior in serum chloride, and no significant changes in serum potassium or in serum protein. Adrenocortical extract often prevented or reduced this drop in serum sodium during their treatments. Their experimental condi-

tions and ours were almost totally different, so that too much weight cannot be placed upon discrepancies in results, such as their drop in serum sodium and our rise.

d. *Chemical changes in sweat.* Before discussing the data on sweat, we have to consider factors influencing its composition. Johnson, Pitts and Consolazio (1944) have suggested that the chloride concentration in sweat is affected by 3 primary factors: (1) a peripheral one correlated with skin temperature; (2) a central one correlated with rectal temperature and rate of sweating and (3) individual idiosyncrasy. With increasing rectal temperature and rate of sweating the sweat chloride increases. A further increase is produced by rise in local skin temperature, and a decrease by lowering the local skin temperature. There may be consistent differences between subjects even when their skin tempera-

TABLE 3

*Serum chloride, sodium, potassium and non-protein nitrogen as affected by administration of water, adrenal cortical extract or both*  
(For experimental conditions see text)

EXPERIMENTAL CONDITIONS	SERUM CHLORIDE			SERUM SODIUM			SERUM POTASSIUM			SERUM NON-PROTEIN NITROGEN		
	1st hr.	3rd hr.	Δ	1st hr.	3rd hr.	Δ	1st hr.	3rd hr.	Δ	1st hr.	3rd hr.	Δ
	meq./l.			meq./l.			meq./l.			mg./100 ml.		
Subject R. W.												
No water.....	104	107	+3	139	150	+11	5.0	5.3	+0.3	30	36	+6
No water, cortex..	106	108	+2	143	150	+7	5.6	5.2	-0.4	36	42	+6
Water alone.....	104	102	-2	142	142	0	4.9	5.4	+0.5	28	35	+7
Water and cortex..	103	102	-1	137	141	+4	5.3	5.8	+0.5	28	39	+11
Subject J. S.												
No water.....	107	106	-1	143	149	+6	5.0	5.0	0	36	46	+10
No water, cortex..	107	108	+1	140	149	+9	5.1	4.8	-0.3	29	52	+23
Water alone.....	102	102	0	139	142	+3	5.4	4.7	-0.7	34	42	+8
Water and cortex..	104	104	0	140	139	-1	4.9	4.9	0	34	30	-4

tures, rectal temperatures and rates of sweating are identical. The present experiments provide information on sweat sodium, potassium and non-protein nitrogen in addition to sweat chloride and suggest that sodium behaves like chloride, but potassium does not. Reference should be made to table 4 for the changes in composition of sweat in relation to skin temperature, to table 1 for the corresponding rates of sweating and rectal temperatures, and to table 3 for the corresponding serum levels. Five chief conclusions may be reached. First, chloride in general showed the changes expected from the interplay of skin and rectal temperatures and rate of sweating. Second, sodium was present in concentrations almost identical to chloride, and showed the same correlations with skin and rectal temperatures and with rate of sweating. Third, in contrast with sodium and chloride, potassium invariably decreased as work was prolonged, and showed no correlation with the factors discussed above. Fourth, non-



protein nitrogen showed no consistent correlation except as it tended to increase as work progressed. Finally, although the results are not so clear cut as one would desire, administration of adrenal cortex was associated with a decrease in sweat chloride and sodium and an increase in sweat potassium, even at equivalent skin temperatures, rectal temperatures and rates of sweating. This was most striking in the case of chloride and sodium in the third hour of work. By analogy with its effect on the kidney, it might be expected that adrenal cortex would lower the sweat sodium and raise the potassium, and Edelman, Mahanna, Lewis, Thatcher and Hartman (1943) have reported such an effect in the case of sodium.

e. *Changes in overall chemical balance.* Thorn, Garbutt, Hitchcock and Hartman (1937) have shown that adrenocortical extract produces in normal subjects

TABLE 4

*Sweat chloride, sodium, potassium and non-protein nitrogen and skin temperature as affected by administration of water, adrenal cortical extract or both*

(For experimental conditions see text)

EXPERIMENTAL CONDITIONS	SWEAT CHLORIDE			SWEAT SODIUM			SWEAT POTASSIUM			SWEAT NON-PROTEIN NITROGEN			SKIN TEMPERATURE UNDER GLOVE		
	1st hr.	3rd hr.	Δ	1st hr.	3rd hr.	Δ	1st hr.	3rd hr.	Δ	1st hr.	3rd hr.	Δ	1st hr.	3rd hr.	Δ
	meq./l.			meq./l.			meq./l.			mg./100 ml.			°F.	°F.	°F.
Subject R. W.															
No water.....	47	56	+9	50	59	+9	6.6	6.5	-0.1	24	35	+11	99.1	100.6	+1.5
No water, cortex.....	38	44	+6	41	46	+5	7.4	6.8	-0.6	29	34	+5	98.9	100.1	+1.2
Water alone.....	44	43	-1	49	47	-2	7.0	5.6	-1.4	32	30	-2	98.0	98.0	0
Water and cortex.....	35	38	+3	38	40	+2	7.0	6.1	-0.9	23	34	+11	98.1	98.2	+0.1
Subject J. S.															
No water.....	60	83	+23	61	85	+24	5.5	6.4	+0.9	30	36	+6	98.9	100.1	+1.2
No water, cortex.....	67	69	+2	69	78	+9	6.5	5.4	-1.1	33	35	+2	98.8	99.1	+0.3
Water alone.....	60	73	+13	62	76	+14	6.0	5.6	-0.4	19	34	+15	96.9	97.4	+0.5
Water and cortex.....	51	56	+5	51	58	+7	7.0	5.9	-1.1	38	50	+12	97.9	98.5	+0.6

a marked reduction in the daily excretion of sodium and chloride and an increase in the excretion of potassium. In the present experiments, the daily urinary excretion of potassium was invariably increased by as much as 1.7 grams when cortical extract was administered, but there was no consistent change in sodium, chloride or nitrogen. Complete balance sheets are not presented owing to the large and variable daily loss of sweat and the difficulty of calculating accurately the composition of total body sweat from analyses on a sample collected in one relatively small area (see Dill, 1938, and Mickelsen and Keys, 1943).

DISCUSSION. We may conclude for practical purposes that the administration of adrenocortical extract to normal young men even in large doses is without benefit upon their capacity to work hard in the heat, in comparison with the striking effects of ingesting plain water. The present experiments allow no conclusions concerning possible effects on acclimatization.

The evidence presented is suggestive that adrenocortical extract may affect the level of electrolytes in the sweat. The sweat glands are known actively to secrete lactate (Dill, 1938; Mickelsen and Keys, 1943). Comparison of serum (table 3) and sweat (table 4) levels at equivalent experimental times shows that the glands apparently secrete potassium actively and impose a barrier to the passage of sodium and chloride. Administration of adrenocortical extract may enhance the secretion of potassium and increase the barrier to sodium and chloride.

#### SUMMARY

1. The effects of large doses of extract of adrenal cortex were studied in healthy young men living on a constant adequate diet and marching  $3\frac{1}{2}$  hours daily in moist heat.

2. In comparison with the striking influence of water drunk during work the extract had no effect beneficial or otherwise on the subjects' performance or feelings.

3. The adrenal cortical extract had no consistent effects on the following physiological or chemical functions in the course of marching or during the whole day: *a*, heat balance as measured by pulse rate, rectal temperature, rate of sweating and skin temperature; *b*, respiratory exchange as measured by pulmonary ventilation, oxygen consumption and carbon dioxide excretion; *c*, blood pressure, either systolic or diastolic; *d*, serum chloride, sodium, potassium or non-protein nitrogen; *e*, daily urinary excretion of sodium and of chloride.

4. On days when extract was injected, the urinary excretion of potassium was increased.

5. Suggestive but not unequivocal evidence was obtained that the extract lowered the concentration of chloride and sodium in the sweat and raised the concentration of potassium.

6. A previous hypothesis concerning the normal regulation of sweat chloride is extended to sweat sodium. Both are present in sweat in almost identical concentrations usually much lower than in the serum and appear to be correlated with 3 important factors: *a*, personal idiosyncrasy; *b*, a central factor measured by rectal temperature and rate of sweating, and *c*, a peripheral factor associated with skin temperature. The general level rises with increasing rectal temperature and rate of sweating. Superimposed on this general level are fluctuations associated with changing local skin temperature. In contrast with sodium and chloride, potassium apparently cannot be included in the above hypothesis and is actively secreted by the sweat glands.

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# INFLUENCE OF PARA-QUINONES ON BLOOD PRESSURE IN HYPERTENSIVE RATS

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Some evidence supports the view that hypertension observed in renal ischemia is due to faulty deamination of amino acids. By the action of decarboxylases pressor amines are formed as intermediary products in the metabolism of certain amino acids. In the kidney with normal blood supply these amines are deaminized to inert substances through the action of amino oxidases. In the ischemic kidney, however, the latter enzymes are not properly active and so pressor amines accumulate and may probably be the main cause of the hypertension observed under such conditions. Soloway and Oster (1) have shown that some pressor amines can be inactivated in vivo and in vitro by the action of certain quinone precursors and Friedman, Soloway, Marrus and Oppenheimer (2) demonstrated that 3 p-quinones, e.g., p-xyloquinone, trimethylquinone and thymoquinone, have well-defined blood pressure-reducing qualities in hypertensive rats with experimental cellophane perinephritis. Schafer (3) found that p-xyloquinone also reduces the high blood pressure in neurogenic hypertension in dogs made hypertensive by section of the carotid sinus and aortic depressor proprioceptor nerves and Schwarz and Ziegler (4) proved that 2-methyl-1,4-naphthoquinone effectively reduces the blood pressure in hypertensive rats with silk perinephritis. The latter also stated that a hydroquinone compound, e.g., 2-methyl-1,4-naphthohydroquinone tetrasodium diphosphate did not influence the blood pressure under such conditions. Some other p-quinones tested by Friedman et al. do not have any influence on the blood pressure in experimental hypertension and it was not clear just why some of the quinones are effective while others are not. Too few compounds were investigated to permit correlation of antipressor properties and chemical structure. Therefore, the influence of a series of p-quinones and hydroquinones on the blood pressure of hypertensive rats was determined. It was thought perhaps possible, thus, to obtain some clear picture of the metabolic disturbances in experimental hypertension.

**METHODS.** Young rats of about 150 to 200 grams were made hypertensive by wrapping both kidneys in silk; the operation on both sides was performed in one stage. The blood pressure was determined by the plethysmographic method of Grollman, Harrison and Williams (5). Rats were considered suitable for testing when the blood pressure level had been elevated and stable for some time. The best assays were obtained 6 to 8 weeks after operation. Daily readings were

<sup>1</sup> The authors wish to express their gratitude to the American Philosophical Society for financial support from the Daland Fund.

made for one week prior to, during, and, if possible, immediately following administration of the agent to be tested.

Thirteen of the 18 compounds used in these experiments were prepared in our own laboratory. Great care was taken in securing pure preparations and stable solutions. The melting points of the p-quinones and hydroquinones used are given in the table.

The doses used for the assays were selected in such a way that they might, as far as possible, correspond to the lowest effective dose. Some experiments were also performed with higher doses in order to permit an estimate of the relative effectiveness of different compounds. Only such compounds were considered to be effective anti-pressors which, without producing any noticeable toxic effects, showed 1, a fall to below 150 mm. Hg, and 2, a maximal drop of at least 30 mm. Hg in the average of the experiment used in the particular assay. With the compounds found to be ineffective, assays were sometimes prolonged or higher doses were given in order to improve the accuracy of the results. The total number of assays amounted to about 90. Of this number about 22 were used for the preliminary establishment of doses and had to be discarded for different reasons. This evaluation left us at least 3 to 5 test animals for any more or less important assay. In 4 assays when only 2 animals were available for each test, the results were so obvious that another series of experiments did not seem necessary.

**RESULTS.** The table shows the results of the assays of 14 p-quinones and 4 hydroquinones. It also summarizes these results and demonstrates which compounds were found to be effective depressors or ineffective substances, and makes it clear that with the exception of naphthoquinone alone, all our studies point to the fact that there exists a definite structural relationship among the compounds which are considered to be effective depressors in hypertensive rats.

*Table 1.* All the effective p-benzoquinones and p-naphthoquinones possess at least 1 hydrogen and 1 alkyl radical on the oxidized benzene ring while the inactive compounds do not possess one or the other radical. This is shown in that toluquinone and 2-methyl-1,4-naphthoquinone are effective depressors, while duroquinone, benzoquinone, anthraquinone and 2,3-dimethylnaphthoquinone are completely ineffective. The essential position of the hydrogen and the alkyl group becomes clear when one imagines the oxidized benzene ring as having been divided into half in the vertical direction. All the effective compounds show the hydrogen and the alkyl radical on the same half of the ring while the ineffective compounds may have the same alkyl radical and the hydrogen on different halves of the ring. This is demonstrated by the fact that the m- and p-xyloquinones are effective while 2,3-dimethylbenzoquinone (o-xyloquinone) is ineffective. The introduction of a second or third alkyl radical into the active benzene ring does not principally change the effectiveness of the compound so long as both hydrogen and alkyl groups are in proper position on the benzene ring. This assumption is proved by the fact that 2,5-dimethyl-p-quinone and 2,6-dimethyl-p-quinone show almost the same effectiveness and thymoquinone and trimethylquinone (Friedman et al.) show remarkable depressor activity. The influence

of differences in the composition of the alkyl radicals upon the depressor activity of the p-quinones can not be discussed conclusively since the great majority of our quinones were methyl substituted. It can, however, be stated that while the 2,5-dimethyl-p-quinone shows very great depressor activity, 2,5-diethyl-p-quinone seems to be completely ineffective when given in equimolecular amounts.

TABLE 1

*The effect of p-quinones and hydroquinones on elevated blood pressure*

COMPOUNDS		M.P. C.°	DAILY DOSE	NO. OF RATS	TOTAL NO. OF DAILY INJECTIONS	AVERAGE BLOOD PRESSURE IN MM. Hg				ACTIVITY
						Be- fore	During 3-4 days of injections	During period of injections	During injections Lowest pressure of any day	
1,4-Benzoquinone	Quinone	113-114	2	2	4	162	173	173	163	Inactive
1,4-Naphthoquinone	$\alpha$ -Naphthoquinone	125-126	2-4	5	4	161	129	129	102	Depressor
9,10-Anthraquinone	Anthraquinone	280-282	5-10	2	4	182	167	167	160	Inactive
1,2,3,4-Tetrahydro- naphthoquinone-5,8		55.5-56	5	3	5	166	180	184	166	Inactive
2-Methylbenzoquinone	Toluquinone	65-67	5	2	4	175	143	143	116	Depressor
2,5-Dimethylbenzoquinone	p-Xyloquinone	124-125	5	4	3	168	126	126	113	Depressor
2,6-Dimethylbenzoquinone	m-Xyloquinone	72-73	5	3	3	161	123	123	104	Depressor
2-Methyl,5-isopropyl- benzoquinone	Thymoquinone	43-44	5	3	8	162	150	146	130	Depressor
2,3-Dimethylbenzoquinone	o-Xyloquinone	55-56	5	2	4 & 9	157	152	152	145	Inactive
2,5-Diethylbenzoquinone		76-77	5.5	3	3	191	170	170	162	Inactive
2,3,5,6-Tetramethyl- benzoquinone	Duroquinone	112-113	5	3	4	157	175	175	162	Inactive
2-Methyl-1,4-naphthoquinone	Menadione	105-107	7-13.5	5	10	168	153	144	118	Depressor
			5-10	9	4	167	144	144	129	
2,3-Dimethyl-1,4-naphthoquinone		123-124	15	3	10	170	168	162	145	Inactive
2-Ethyl-1,4-naphthoquinone		87-88	15-20	3	12	154	145	139	122	Depressor
	Toluhydroquinone	124-125	5	2	5	166	167	169	162	Inactive
	m-Xylohydroquinone	149-150	5	5	4	163	171	171	158	Inactive
	Thymohydroquinone	144-145	5	3	5	155	164	164	155	Inactive
	*Synkayvite		5-10	5	4	170	174	174	165	Inactive

\* 2-Methyl-1,4-naphthohydroquinone tetrasodium diphosphate.

These findings are somewhat contradicted by the results of our studies on 2-ethyl-1,4-naphthoquinone, which may show pressure-lowering qualities similar to those found with 2-methyl-1,4-naphthoquinone. The former compound, however, requires relatively higher doses and it is not beyond any possibility that some 2-ethyl-1,4-naphthoquinone may be transformed into 2-methyl-1,4-naphthoquinone in the body of the animal (Almquist, 6). Substituted benzoquinones and substituted naphthoquinones may produce somewhat similar

depressor effects. The only difference worth mentioning here is a difference in the time each of the compounds requires for its optimal effectiveness. The naphthoquinones seem to act more slowly than the benzoquinones and it is not thought that these differences are due to a different uptake by the tissues of the animals. Similarly where the effect of the benzoquinones lasts for two to three days, before returning to the previous high level, Menadione and 2-ethylnaphthoquinone may act for a period approximately three times as long. 1,4-Naphthoquinone<sup>2</sup> is the only effective substance tested so far which does not possess all the structural qualities believed to be essential for the depressor action of the p-quinones. It should be pointed out, however, that naphthoquinone produces some toxic effects and that the difference between the lethal and the pressure-lowering dose is relatively small.

The studies on the influence of hydroquinones in hypertensive rats were started in the belief that hydroquinones might have blood pressure-lowering qualities similar to the quinones with the same type of structure. Such an assumption seemed to be justified since under different experimental conditions, e.g., in vitamin K deficiency, 2-methyl-1,4-naphthohydroquinone tetrasodium diphosphate is about as active as 2-methyl-1,4-naphthoquinone. Moreover, it was to be expected that substances so easily oxidizable as the hydroquinones should readily be oxidized in hypertensive animals and thus give the same type of quinones capable of lowering the blood pressure. A study of the table summarizing results in four different hydroquinones shows that none of the hydroquinones produces even the slightest lowering of the elevated blood pressure. Since all the corresponding quinones were of the active depressors (table) one may assume with some justification that exceedingly little if any oxidation of these hydroquinones could have taken place in our animals with experimental hypertension. It would, of course, be of the utmost importance to know whether, and to what extent hydroquinones of the type used are oxidized to quinones by the normal organism or whether the disturbance of the oxidation of certain hydroquinones may be a characteristic of hypertensive conditions. With the evidence now at hand no conclusive statement can be made. Very recent studies of Richert (7), however, show that one of the hydroquinones used, the 2-methyl-1,4-naphthohydroquinone tetrasodium diphosphate must readily be oxidized in normal animals. Therefore, at least for the latter hydroquinone, there seems to be some basis for the assumption of a disturbed oxidation in experimental hypertension.

The exact mechanism of the depressor activity of the p-quinones is still so much in the dark that it doesn't seem to be very useful to discuss the many possibilities. It may be pointed out that Friedman et al. found that several benzoquinones which lower the blood pressure in hypertensive animals do not affect the blood pressure of normal, non-hypertensive animals when given in the same doses, a fact we confirmed with 2-methyl-1,4-naphthoquinone. Also, that there seems

<sup>2</sup> While this paper was being written, a paper (B. S. Oppenheimer, S. Soloway and B. E. Lowenstein, J. Mt. Sinai Hospital, N. Y., 11: 24, 1944) came to our attention, in which we noted that we had arrived at the same conclusions regarding  $\alpha$ -naphthoquinone and 2,3-dimethylnaphthoquinone as these authors did.

to be no parallelism between the depressor activity and vitamin K activity or between the depressor activity and the electropotentials of the particular compounds.

# SUMMARY

1. The effectiveness of p-quinones in reducing the blood pressure of perinephritic hypertensive rats was found to depend on a certain chemical structure. The effective compounds possess 1 hydrogen and 1 alkyl radical on the same half of the oxidized benzene ring when the ring is imaginarily divided in the vertical direction. Compounds which do not have these essential groups and structure are ineffective. The composition of the radical may be of some importance. p-Benzoquinones and p-naphthoquinones show principally the same depressor activity except for the different time they need to reach their maximal effectiveness.

2. The hydroquinones with the same alkyl substituents and the same type of structure are completely ineffective. Since hydroquinones may be oxidized to quinones in the body of normal animals, the ineffectiveness of hydroquinones on elevated blood pressure in hypertension suggests disturbed oxidation in hypertensive conditions.

3. The differences in the depressor activity of different p-quinones seem not to be related to the different vitamin K activity or to the different electropotential of these compounds.

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# EFFECTS OF ACUTE HEMORRHAGE AND OF SUBSEQUENT INFUSION UPON THE BLOOD VESSELS AND BLOOD FLOW AS SEEN IN THE MESENTERIES OF ANESTHETIZED DOGS<sup>1</sup>

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In these experiments the effect of hemorrhage and of infusions upon the blood vessels and blood flow of the mesenteries of 13 anesthetized dogs was observed in transparent intestinal mesenteric chambers such as described by Zintel (1) for the rabbit. The original rabbit type of intestinal mesenteric chamber has been modified to fit the dog by the present authors (2).

**METHOD.** The smaller vessels of the mesentery were examined and photographed at magnifications of 200 and 400 diameters. The arterioles, capillaries, and venules were clearly visible, and especially their walls were distinctly seen. So was the blood flowing through them. The larger vessels of the mesentery were studied at magnifications of 30 diameters with a binocular dissecting microscope. Measurements were made with an ocular micrometer of the diameters of both the smaller and the larger vessels before and after hemorrhage. In addition to single photographs, motion pictures were taken. Arterioles smaller than  $24\mu$  in diameter were not studied. Observations on the behavior of such smaller muscular vessels during acute hemorrhage have been previously described by Zweifach, Lowenstein and Chambers (5).

The hemorrhages were produced as described by Kohlstaedt and Page (3) until the pressure fell to approximately 30 to 40 mm. Hg. It was kept at this level for  $\frac{1}{2}$  to  $\frac{3}{4}$  of an hour by further removal of blood, or by returning blood, according to the need.

Six of 11 dogs<sup>2</sup> were not treated following the period of post-hemorrhagic hypotension, and all of them died within 1 to  $1\frac{1}{2}$  hours after the onset of the hemorrhages. Five were given infusions and survived for three hours, after which they were sacrificed.

The rate of blood flow was determined by direct observation with the microscope, usually at 200 magnifications. No attempt was made to measure the actual velocity of blood flow, but only to contrast by observation the rate of flow following hemorrhage with the control rate.

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<sup>2</sup> We are grateful to Dr. Kenneth Kohlstaedt and Mr. Clifford Wilson for allowing us to use, during the course of their experiments, 5 of their dogs in these infusion experiments, and for their assistance.

The dilution of the blood following hemorrhage could be seen directly with the microscope. This could be readily detected because of the reduced rate of flow which followed hemorrhage.

In all of the dogs the blood pressure was measured by carotid cannulation and recorded by a kymograph.

The dogs weighed between 10 and 14 kilos, and were anesthetized by 30 mgm./kgm. of pentobarbital injected intraperitoneally (11 cases) or intravenously (2 cases),<sup>3</sup> 1 to 3½ hours prior to bleeding.

EXPERIMENTAL. 1. *Control observations.* The mesenteric blood vessels in each of the 13 dogs were studied for periods of from 1 to 3 hours preceding the onset of hemorrhage. The larger arteries studied had diameters of from 0.39 to 1.2 mm., the larger veins from 1.0 to 2.1 mm., the arterioles from 24 to 65 $\mu$ , and the venules from 16 to 120 $\mu$ . No record was made of caliber changes of arterial muscular vessels less than 24 $\mu$  in diameter. During the control periods, none of these vessels changed appreciably in diameter.

In two additional dogs no hemorrhages were produced, but instead, the vessels were studied as controls at intervals over a total period of 10 and 11 hours respectively. This was longer than the duration of any of the hemorrhage experiments, yet no appreciable changes were observed in the diameters of the vessels, or in the rates of flow through them. The flow was usually so rapid through arterioles having diameters between 40 and 60 $\mu$  that its direction was difficult to determine when the vessels were examined at a magnification of 200 diameters. In the venules of corresponding size it was frequently almost as fast. It never was slow or pulsatile.

2. *The effect of hemorrhage upon the blood vessels.* The removal of 1 to 2 per cent of the body weight of blood caused no appreciable change in the diameters of the mesenteric blood vessels. Following removal of larger amounts (2.5–5.5 per cent), the larger arteries (0.39–1.2 mm. diameter) constricted 20 to 60 per cent and the smaller ones (24 to 65 $\mu$  diameter) usually to a like degree, though in some cases slightly more. An arteriole illustrating such constriction is shown in figures 1, 2 and 3. The walls of the arteries and arterioles became noticeably thicker at the time of their constriction (figs. 2 and 3).

Studies of the arterioles 24 to 65 $\mu$  diameter showed that in cases in which the dogs died as a result of the hemorrhages, relaxation of the constricted state occurred only a short time before death. In some instances relaxation was to the original diameters; in others it was not. Thus in one dog an arteriole having a control diameter of 48 $\mu$  constricted to 0.5 its control diameter when the pressure reached 30 mm. Hg. Eleven minutes before death it had relaxed to its control diameter. In another dog an arteriole having a control diameter of 54 $\mu$  constricted to 0.44 of this diameter as a result of hemorrhage. Sixteen minutes before the dog died, it relaxed partially to 0.66 its control diameter. Death regularly occurred in untreated dogs shortly after the onset of arteriolar relaxa-

<sup>3</sup> In the case of these two dogs the hemorrhages were produced by Dr. William M. Parkins, with the assistance of Mr. L. H. Saxe and Miss Jeanne Evans, to all of whom we are indebted.

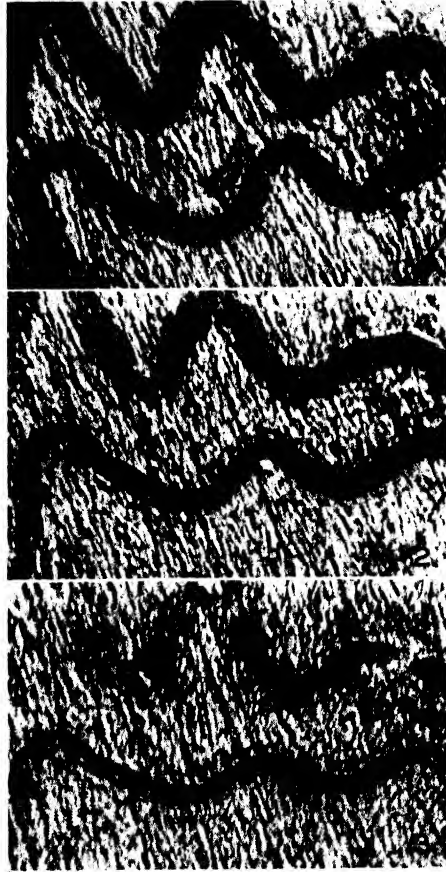


Fig. 1. Photomicrograph of an arteriole, *A*, and venule, *V*, in a portion of a dog's mesentery inclosed within an intestinal-mesenteric chamber. This photomicrograph was taken 2 hours and 18 minutes after installation of the chamber, and shows the control appearance of the blood vessels. The actual diameter of the arteriole was  $36\mu$ . The blur in the arteriole is due to rapid blood flow. The difference in blurring in the venule indicates a slightly slower flow than in the arteriole, but still a rapid one. The mean blood pressure was 108 mm. Hg.  $\times 110$ .

Fig. 2. Photomicrograph of the same vessels as in figure 1, after removal of 2.5 per cent of the body weight of blood. The pressure when this photomicrograph was taken was 54 mm. Hg. The arteriole has constricted approximately 30 per cent, but the diameter of the venule has not changed appreciably. The flow in the arteriole at this time was definitely slower than the control flow, but still moderately rapid. The flow in the venule was much reduced, as shown by the incompleteness of the blurring.  $\times 110$ .

Fig. 3. Photomicrograph of the same vessels as in figure 1, taken following removal of blood to the amount of 3.5 per cent of the body weight. This reduced the pressure to 30-35 mm. Hg, at which level it had been maintained for 18 minutes before this photograph was taken. The arteriole is constricted, but the venule is not. The flow in the arteriole was slow and pulsatile at this time; in the venule there was hardly any movement of blood. In the venule the erythrocytes have formed clumps, which are made up of groups of cells in rouleaux. There is free plasma between the clumps.  $\times 110$ .

tion. Reference is intended here only to arterioles having diameters of  $24\mu$  or more, since no attempt was made to study smaller ones. Within a minute or so following failure of the respiration intense constriction of the muscular vessels was observed.

In many instances the larger veins (1.0 to 2.1 mm.) also constricted following hemorrhage, the degree of constriction being from 12 to 43 per cent of their control diameters.

The venules ( $16$  to  $120\mu$ ) did not constrict. In some cases their diameters did not change (cf. figs. 1, 2 and 3). In others they dilated to 1.1 to 1.2 times their control diameters after prolonged reduction of flow.

The capillaries were not observed to constrict or to dilate.

3. *The effect of hemorrhage upon the blood flow.* The initial removal of 1 to 2 per cent of body weight of blood had little immediate effect upon the blood flow, except for a temporary slowing during the actual time blood was being drawn. Removal of blood in sufficient quantities to cause the pressure to fall to about 90 mm. Hg produced a slight slowing in flow in all vessels. Removal of blood in sufficient quantities to cause the pressure to fall to between 40 and 30 mm. Hg was usually followed by marked slowing in flow. Figures 1, 2 and 3 show the effects of removal of various amounts of blood upon the blood flow.

At 30 mm. Hg, a pressure produced in these experiments by removal of 3.5 to 5.5 per cent of body weight of blood, the arterial flow was in most instances slow and pulsatile; individual red blood corpuscles could readily be observed even in the arterioles, in marked contrast to the control condition, in which the flow was so rapid that even its direction of movement was not obvious. Sometimes at this low pressure the arteriolar flow was intermittent. Stasis was frequently present in some of the venules. Where flow in the venules was present, it was extremely slow. The capillaries contained less blood than normal and the capillary bed was ischemic. In some cases there was temporary reversal of the normal direction of flow in the venules and capillaries—the corpuscles in these cases moving slowly toward the arterioles.

The effect of respiration upon the flow in the arterioles and venules was frequently apparent at blood pressures of approximately 30 mm. Hg. During expiration the flow in the venules stopped, whereas during inspiration it moved forward slowly. The effect of respiration upon the arteriolar flow was the opposite. Thus inspiration decreased arteriolar flow, but increased venular flow.

Some variation was observed in different animals, in rates of flow at the 30 mm. level of blood pressure. Blood flow at this pressure was usually extremely slow, or, in many of the vessels, absent. In none was it rapid. In two of the dogs, however, moderate flow persisted until the pressure dropped to the low level of 18 mm. Hg.

Wide excursions in arterial pressure recurring in waves were sometimes observed following hemorrhage. When the mean pressure at which such waves occurred was low, the arteriolar flow was seen to stop at the bottom of such waves (usually when the pressure at the bottom of the wave was 30 mm. Hg or below) and to be faster at the top.

4. *The effect of hemorrhage upon the corpuscle/plasma ratio.* During control periods, the red blood cells almost completely filled the venules, there being only a thin layer of plasma between them and the vessel walls. Within 15 minutes following removal of large amounts of blood (usually 4 to 5.5 per cent of the body weight) the plasma/corpuscle ratio became much increased. Fewer red blood cells than normal were present within the plasma at such times, and these cells were seen to be farther apart than usual. Thus the hemodilution that is known to occur in dogs after severe hemorrhage was observed in the vessels directly with the microscope.

5. *The effect of hemorrhage upon the color of the intestine and tongue.* At arterial pressures of 30 to 40 mm. Hg the intestine within the chamber became bluish-red, in contrast to its normal pink color during control periods. At such low pressures the tongue became the same bluish-red color, and, like the intestine, appeared cyanotic.

6. *The effect of infusion at the end of the hypotensive period.* In the 5 dogs infusions were given after the pressure had been kept at 30 to 35 mm. Hg for  $\frac{1}{2}$  to  $\frac{3}{4}$  of an hour. In one, all of the blood that had been removed was given back. In the other four, heterologous albumin was infused instead, the amount being that calculated to restore the osmotic pressure of the blood to its prehemorrhage level. The total volume given was less than 100 cc. in all instances. In the dog in which all of the blood withdrawn was re-infused, the removal of 4.5 per cent of body weight of blood caused the pressure to fall to 30 mm. Hg. At the end of the half-hour period during which the pressure was maintained at this level, stasis was present in many of the venules, and blood flow was extremely slow in all of the other vessels. The arteries and arterioles were constricted. The intestine and tongue were bluish-red.

Intra-arterial infusion by the method described by Kohlstaedt and Page (3) was started. Within 5 minutes blood flow had begun in many of the vessels in which there had been stasis, and it had increased in rate in others; within 10 minutes the pressure had risen to 70 mm. Hg and the blood flow was moderately rapid. At the end of 25 minutes all of the blood had been given back. At this time the blood flow had returned to the control rate. The arterioles were no longer constricted. The intestine and the tongue were pink. Normal blood flow, vessel size, and color of the intestine persisted for at least 3 hours. The dog was then bled again. Removal of only 3.25 per cent caused the arterial pressure to fall to 30 mm. Hg, from which level it gradually declined, and fell to zero one hour after the onset of the hemorrhage. Following the second hemorrhage, the blood vessels and blood flow behaved as they did following the first, except that in addition there was partial arterial relaxation 10 minutes before death.

Isosmotic albumin was given at the rate of 16 cc. per minute by intravenous drip to 4 dogs subjected to hemorrhage. Following this, arterial pressure rose and blood flow once again became rapid. Likewise, the color of the intestine and tongue changed from bluish-red to pink. The arteries and arterioles relaxed to 0.8 to 0.9 of their control diameters. The least rise of blood pressure was to 70 mm. Hg and the greatest to 118 mm. Hg.

In the first experiment, the dog and the albumin were both kept at room temperature. In the second, the dog was kept in a tent at 45°C. and the albumin infused was also at 45°C.

The greatest rise in pressure (to 118 mm. Hg) and the most rapid rate of flow following albumin infusion was observed in the case in which the dog and the albumin were kept at room temperature. The data suggest that albumin infusion at the end of a period of post-hemorrhagic hypotension of the degree and length described above restores blood flow, causes constricted arteries and arterioles to relax, the blood pressure to rise, and the intestine and tongue to return to approximately their normal colors.

**DISCUSSION.** Severe hemorrhage causes constriction of both the large and small arteries and of the larger veins, but not of the venules or the capillaries of the dog's mesentery.

Constriction of the arteries and arterioles has now been observed in a number of animals following shock induced by various methods. Thus it has been seen in the mesentery of cats following application of limb tourniquets (4) and following burns (2), in the skin and viscera of rats after hemorrhage (5) and in the rabbit's ear following intestinal manipulation (6).

Freeman, Shaffer, Schechter and Holling (7) found that following hemorrhages the blood flow in the limb as measured by a plethysmographic method is reduced more in normal than in sympathectomized cats, suggesting that vasoconstriction may be a factor in reducing peripheral blood flow in hemorrhage.

Page (8) reports that a vasoconstrictor substance differing from angiotonin appears in the blood within 5 minutes following rapid removal of 2 to 7 per cent of the body weight of blood, in a series of experiments in which no observations of the blood vessels were made. It is significant to note that in the present experiments vasoconstriction was actually observed at a comparable period following hemorrhage.

Hamilton and Collins (9) have presented evidence that an angiotonin-like vasoconstrictor appears in the blood following hemorrhage, and Huidobro and Braun-Menendez (10) report that renin can be detected in the systemic blood of dogs following removal of 4 per cent of the body weight of blood.

In the majority of dogs in the present experiments, removal of about 5 per cent (sometimes more) of the body weight of blood from dogs anesthetized with pentobarbital lowered the blood pressure to approximately 30 mm. Hg, and was invariably fatal. In a few dogs removal of as little as 3.5 per cent was sufficient to lower the blood pressure to this level. In all cases the blood flow became slowed, but an occasional dog maintained a moderately good flow even at this low pressure. Thus blood pressure is not a constantly reliable index of peripheral blood flow.

An interesting example emphasizing the unreliability of blood pressure alone as an index of peripheral blood flow is that described by Page, Kohlstaedt and Taylor (11) in which a man had taken arsenic for suicidal purposes. Despite the fact that arterial pressure fell to about 38 mm. mean pressure in the recumbent position, he felt well and was able to rise and walk without difficulty. Flow to the peripheral tissue was not appreciably reduced.

A reduction in blood flow similar in degree to that found in the present acute hemorrhage experiments was also observed in the mesenteries of cats during shock produced by venous tourniquets and in burns (Page and Abell, 4; Abell and Page, 2). More recently Chambers, Zweifach and Lowenstein (12) reported slowing of blood flow concurrently with fall in blood pressure in the mesoappendix of rats traumatized in the Noble-Collip drum. The terminal stage was characterized by stagnation, especially in the venous portion of the capillary bed, and by back flow from the veins. Levinson and Essex (6), using the transparent chamber technic developed by Clark, observed reduction in quantity of blood flowing in the vessels in chambers in innervated and denervated rabbits' ears during shock induced by intestinal manipulation.

It is interesting to note that following hemorrhages in rats similar to those produced in the present experiments in dogs, Zweifach, Lowenstein and Chambers (5) observed in the capillary bed an ischemic capillary circulation which was accompanied by vasoconstriction of the muscular vessels and hyper-reactivity to epinephrin. Thus at the time that the large blood vessels of the mesentery are constricted, as shown by the present experiments, the capillary bed shows in the rat a similar type of reaction, that is, one tending to restrict the flow through it. In our experiments, as in theirs, no vasoconstriction or reduction in blood flow in the mesentery occurred until a blood loss of slightly more than 2 per cent of body weight had been reached. This agrees closely with the observations in the rat mesoappendix (5), where no change in flow was observed until a blood loss of 2.0 to 2.5 per cent. The backflow seen in the present dog experiments was also observed in the rat mesoappendix (5). Like the larger vessels in the present experiments, the terminal vessels in the rat remained narrowed and the circulation ischemic to within 10 to 15 minutes of death.

Our observations were limited to the arteries and to arterioles no smaller than  $24\mu$  in diameter. Shortly before death these were seen to relax. This invariably marked the onset of circulatory collapse. A similar relaxation of the smaller arterial muscular vessels was observed by Zweifach, Lowenstein and Chambers (5) in the late stages of circulatory collapse induced by acute hemorrhage in the rat. They likewise observed constriction of the muscular vessels within a short time following cessation of the respiration.

Thus a significant feature of the circulatory collapse following hemorrhage (the collapse which precedes failure of the respiration) appears to be a relaxation of the arteries and arterioles, whereas a significant feature of earlier stages appears to be arterial and arteriolar constriction. A similar relaxation of the initial vasoconstriction occurred in the late stages of shock in cats subjected to venous tourniquets (Page and Abell, 4).

It has recently been reported by us (2) that the change in color of the intestine from pink to bluish-red seen following burns of the body surface is paralleled by a similar change in color of the tongue. In the present experiments, this was also found to be true following hemorrhages. Furthermore, a change in color back to pink occurred in both organs following infusion.

In the four cases in which albumin was infused this was followed by a relaxation of the constricted arteries and arterioles and the restoration of active circulation in the vessels of the mesentery. This persisted as long as studied (3 hrs.) and the changes observed compared favorably with the one case in which whole blood was the infusion medium.

#### SUMMARY

1. The method used in these experiments was to bleed the dogs acutely at short intervals until the pressure fell to 30 to 35 mm. Hg, and to maintain the animal in that state for 30 to 45 minutes. Direct microscopic observations of the vessels in the mesentery of the dog showed that the arteries (0.39–1.2 mm. control diameters) constricted 20 to 60 per cent following removal of 2.5 to 5.5 per cent of the body weight of blood. The arterioles (24–65 $\mu$  diameters) usually constricted to a like degree, though sometimes slightly more. In many instances the larger veins (1.0–2.1 mm.) also constricted, the degree being from 12 to 43 per cent. The venules (16–120 $\mu$ ) did not constrict; in some instances they dilated to 1.1 to 1.2 of their control diameters. The capillaries were not observed either to constrict or dilate. No measurements were made of arterioles having diameters of less than 24 $\mu$ .

2. At hypotensive levels of about 30 mm. Hg, produced in these experiments by the removal of 3.5 to 5.5 per cent of the body weight of blood, the arterial flow was usually slow and pulsatile. Sometimes it was intermittent. Stasis was frequently present in some of the venules. For the most part the capillary bed was ischemic. Some variation occurred, however, and an occasional dog maintained a moderately good flow even at this low level of pressure, whereas in some instances a sluggish flow was present in the mesenteries of some dogs even when the blood pressure was as high as 60 mm. Hg.

3. Following such large hemorrhages, direct observations showed that the corpuscles in the vessels were separated by more than the normal amount of plasma.

4. At arterial pressures of 30 to 40 mm. Hg, the intestine became bluish red, in contrast to its normal pink color during control periods.

5. Albumin infusions given into an artery after the pressure had been kept at 30 to 35 mm. Hg for half an hour restored the blood flow, caused the constricted arteries and arterioles to relax, the blood pressure to rise, and the intestine and tongue to return approximately to their normal colors. This was similar to the changes observed when the blood previously withdrawn was returned.<sup>4</sup>

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# THE RELATIVE ABSORPTION AND UTILIZATION OF FERROUS AND FERRIC IRON IN ANEMIA AS DETERMINED WITH THE RADIOACTIVE ISOTOPE<sup>1</sup>

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It is gradually becoming more generally appreciated that the normal daily requirement of iron is very low, probably not exceeding 2 or 3 mgm. for adult males and is probably not in excess of 10 mgm. per diem in adolescents and adult women. However, during growth, pregnancy, excessive catamenic blood loss or following hemorrhage it is frequently necessary to administer iron. This may be needed to re-establish the normal reserve storage for later exigencies, or actually to supply enough of the needed element for the formation of normochronic red cells and for tissue needs. The literature abounds with expressions of opinion as to the relative merits of organic or inorganic preparations of iron; of soluble versus insoluble forms; and with discussion of the question whether ferrous or ferric salts are more advantageously utilized. Recently we reported some preliminary studies on this latter subject in which it was found that ferrous salts were better absorbed and utilized by the body in time of need (6). We present here further results of these studies indicating that ferrous salts of the compounds investigated are much more efficiently taken up. Whether these observations are to be taken to indicate that in order to be absorbed iron must be converted into the ferrous form we are not prepared to say. Some investigators claim that the limiting factor involved in the absorption of ferric iron is largely the capacity of the gastro-intestinal tract to reduce the element. However, it must be remembered that under any known conditions the overall efficiency of uptake is at best rather low. Only extraordinarily small and therefore therapeutically impractical doses of iron salts result in the absorption of more than 40 per cent of the administered dose (8).

The radioactive isotope of iron lends itself admirably to use in absorption studies since it is possible to distinguish between iron recently introduced into the red cells through feeding of these tagged atoms and that which was already present in circulating hemoglobin. When radioactive iron is found in the peripheral blood it is indisputably the material fed. For the purpose of quantitating results one must make the assumption that all or nearly all of the absorbed iron is used in the construction of new hemoglobin and not to any great extent stored.

<sup>1</sup> This work was made possible through a grant from the Nutrition Foundation.

We believe that under the conditions of iron deficiency there is nearly complete utilization and a minimum of storage (9).

In spite of the distinct advantage of tagged iron for use in these studies it is not a simple matter to obtain clearcut evidence when dealing with patients under ordinary hospital conditions. Some of the difficulties encountered in our work may have been due to errors of nursing technique which are difficult of confirmation. Others were due to the unsuspected presence of masked or quiescent pathology which complicated the picture. Even so, the evidence indicates that the ferrous iron salts were handled more efficiently by these subjects than were the corresponding ferric salts or ferric ammonium citrate. When follow-up experiments were conducted in dogs whose iron deficiency anemia could be maintained under controlled conditions, and in which extraneous pathology could be ruled out subsequently by long observation, the results were indeed striking.

Elsewhere we have reported studies relating to factors involved in absorption of iron by the gastro-intestinal tracts of humans and dogs (1, 5).

**METHODS.** Methods for wet ashing of blood samples, separation and electroplating of the iron and quantitative determination of the radioactivity have already been described (4, 9, 2). The iron isotope used was  $\text{Fe}^{59}$  and beta emission was measured. The material as received from the cyclotron contains very substantial amounts of radioactive impurities including phosphorus, manganese, cobalt, zinc, etc., which must be removed by purification procedures which have been described in detail in another communication (2). Frequent radioactivity determinations were made of the material fed to demonstrate its purity by the adherence of its decay rate to the technical time of 47 days.

Blood samples were taken in either isotonic sodium oxalate or in a dry mixture of potassium and ammonium oxalate. They were centrifuged in calibrated graduated 15 ml. tubes at a speed of at least 2800 r.p.m. for 35 minutes in a large (size 2) International centrifuge. The hematocrit was read and the plasma decanted. The red cells were wet ashed and the concentration of radioactivity<sup>2</sup> per milliliter of packed cells determined. Total mass of red cells in the body was estimated on a basis of body weight assuming 80 ml. of whole blood per kilogram and applying a correction factor of 0.75 to arrive at a value for red cell mass nearer the true value (7). It was assumed that the red cell mass was a linear function of the venous hematocrit (3). Est. red cell mass =  $(80) \times (\text{wgt. in kgm.}) \times (\text{venous hematocrit}) \times (0.75)$ . Total circulating radioactivity =  $(\text{conc. radio iron in red cells}) \times (\text{est. cell mass})$ . Net circulating radioactivity =  $(\text{circ. activity}) - (\text{residual circ. activity from previous feeding})$ .

$$\frac{\text{Net circulating radioactivity}}{\text{Total radioactivity in iron fed} \times 100} = \text{per cent uptake.}$$

The ferric ammonium citrate was prepared by dissolving tagged ferric hydroxide in a minimum of saturated citric acid solution with the aid of heat and

<sup>2</sup> We are indebted to the Bristol-Meyers Company for apparatus used to make some of these measurements.

subsequently adding an excess of ammonia. After driving off the excess  $\text{NH}_3$  on a steam bath the material was dissolved in distilled water and fed.

Ferrous salts were prepared by adding the desired acid to the ferric hydroxide and further adding 3.2 mgm. of cevitamic acid for each milligram of iron present as previously established by analysis. In other instances it was felt advisable to eliminate any reaction which might be attributable to the cevitamic acid and in these instances the iron in the desired salt form was treated with sulfur dioxide gas to reduce it and the excess sulfur dioxide removed by boiling. In the latter procedure the iron was fed immediately after reduction, to prevent excessive oxidation of the ferrous iron by the air.

In nearly every instance the use of ferrous and ferric salts was alternated in order to control the experiments and to obtain the sharpest possible contrast of results. The pertinent hematological data and dosages appear in table 1. Following the first feeding in each case, blood samples were taken on the fourth,

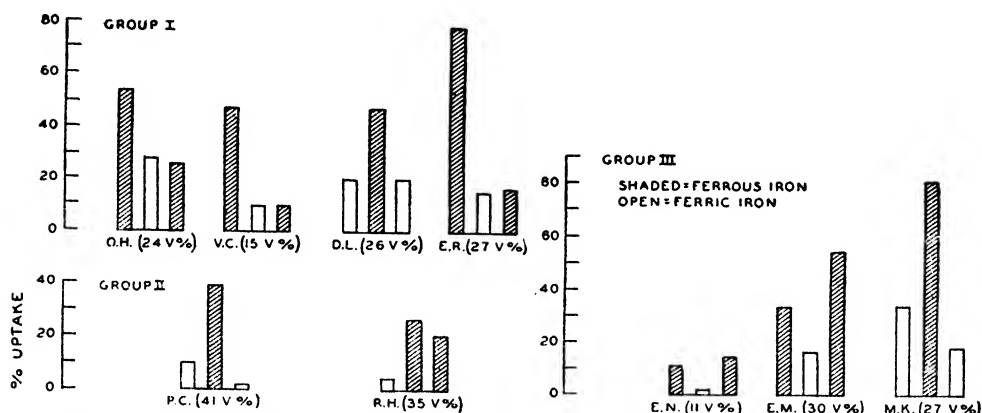


Fig. 1. Relative efficiency of absorption of ferrous and ferric iron in three groups of Hospital patients. V% = packed cell volume.

sixth and eighth days. Utilization is usually incomplete on the fourth day but this sample affords an estimate of the *rate of utilization* (8). The isotope level in the red cells was usually taken as the average found on the sixth and eighth days. The next feeding was always given immediately following the eighth day sample and the level as determined by the sample served as the baseline for determination of additional uptake due to this feeding. Similarly the third feeding was given on the eighth day following the second feeding.

**EXPERIMENTAL OBSERVATIONS.** The patients studied may be divided into three groups. The first comprises four colored patients (fig. 1) who were receiving therapeutic doses of untagged ferrous sulphate orally during the investigation. In each case this was ordered discontinued on the day preceding that on which the tracer dose of radioactive iron was to be administered.

It has been shown (8, 14) that as the dosage of iron is increased the percentage utilization decreases markedly. In this study we are concerned with the percentage utilization of single doses of iron of the order of magnitude of the normal

daily intake. If, through an error, the subject were to receive a therapeutic dose of iron salt near the time of administration of the tracer iron the effect would be to dilute greatly the radioactive iron and we should find a correspondingly smaller percentage uptake. In the case of O. H. there was a discrepancy in the nurses' notes indicating that the usual amount of therapeutic iron may have been administered on the day of the last radio iron feeding (fig. 1, table 1). This might explain the lower uptake of radio iron in this instance. Again in the cases of V. C. and E. R. (fig. 1, table 1), the second dose of tagged ferrous sulphate was handled less efficiently than the first dose, approximating in degree the apparent uptake with the ferric salt. That a similar error might have taken place in these cases also cannot be ruled out. However, it is to be noted that each of these patients was subsequently found to have conditions known to be unfavorable to blood regeneration, the former a carcinoma of the stomach and the latter nephrosclerosis and acute ulcerative colitis. Of these four patients then the only uncomplicated case is that of D. L. who showed well over twice the uptake of ferrous iron as contrasted with the ferric ammonium citrate.

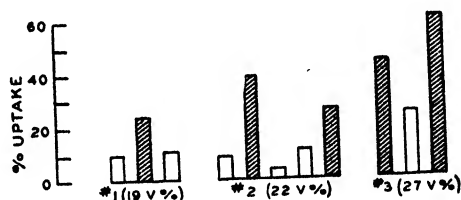


Fig. 2. Relative efficiency of absorption of ferrous and ferric iron in iron deficiency anemic dogs. V% = packed cell volume.

At this stage of the investigation it was felt advisable to study the relative response to feeding of these salts in several standardized dogs. These animals 39-320, 40-149, and 40-115 had been maintained at anemia levels of from  $\frac{1}{4}$  to  $\frac{1}{2}$  normal blood hemoglobin for periods of three, two and one-half and three years respectively, by repeated bleedings. Their responses to iron, induced hemolytic anemia (acetylphenylhydrazin), etc., had been repeatedly shown to be normal and they were in excellent clinical condition throughout and for the several months following these experiments during which they were under observation. They were maintained on a diet of hospital scraps fed once daily. Plasma volume, hematocrit and hemoglobin determinations were carried out at weekly intervals (9), sufficient time being allowed to elapse from any previous bleeding to permit stabilization of the vascular components. The tagged iron, in doses indicated in table 1, was administered by gavage about 16 hours following the last meal and not less than three hours before food was given. Blood samples, as in the case of human subjects, were drawn into isotonic oxalate on the fourth, sixth and eighth days. Red cells were ashed, the iron separated, electroplated, and measurements of radioactivity carried out.

In figure 1 and table 1 are shown the results of eleven experiments in these dogs and in each instance the utilization of ferrous iron was much greater than

TABLE 1

NAME	AGE SEX WT.	DIAGNOSIS	BLOOD PICTURE		RADIO-IRON FEEDINGS			PER CENT OF DOSE AB- SORBED
			Before	After	No.	Dose	Material fed	
	kgm.		RBC Hb. Hct.	millions grams %		mgm.		
V. C.	78 F	Carcinoma of stomach	2.26	3.1	1st	8	Ferrous sulph.	43
			4.2	4.1	2nd	7	Ferric am. cit.	10
			15.1	19.4	3rd	11	Ferrous sulph.	10
D. Lab.	37 F 66	Anteriorly placed uterus with sev- eral irregu- lar masses	4.0	4.2	1st	8	Ferric am. cit.	20
			6.75	9.8	2nd	11	Ferrous sulph.	43
			26.5	34.6	3rd	7	Ferric am. cit.	20
E. R.	33 M 62	Duodenal ulcer	2.84	4.4	1st	8	Ferrous sulph.	78
			7.5	12	2nd	8	Ferric am. cit.	14
			27	39.4	3rd	11	Ferrous sulph.	15
O. H.	38 F 56	Multiple preg- nancy iron deficiency	4.1	5.2	1st	8	Ferrous sulph.	53
			5.7	7.8	2nd	7	Ferric am. cit.	23
			23.8	32	3rd	11	Ferrous sulph.	22
P. C.	36 F 52	Menorrhagia	4.98	4.68	1st	9	Ferric am. cit.	10
			13	12.3	2nd	9	Ferrous sulph.	38
			41	38.5	3rd	12	Ferric am. cit.	2
R. H.	29 F 67	Multiple preg- nancy iron deficiency	3.7		1st	49	Ferric chloride	10
			10.0		2nd	49*	Ferrous chloride	22
			35.0	34.4	3rd	49	Ferrous chloride	20
J. N.	50 M 96	Bleeding duo- denal ulcer	1.86	2.65	1st	15	Ferrous chloride	10
			4.0	5.5	2nd	15	Ferric chloride	2
			11.3	20.6	3rd	15	Ferrous chloride	12
M. K.	36 F 63	Bleeding duo- denal ulcer	3.42		1st	15	Ferric chloride	34
			7.5		2nd	15	Ferrous chloride	80
			27.0		3rd	15	Ferric chloride	18
E. M.	23 F 56	Metrorrhagia	3.8	2.9	1st	15	Ferrous chloride	34
			8.2	5.7	2nd	15	Ferric chloride	17
			30.3	25.0	3rd	15	Ferrous chloride	55
DOGS								
39-320		Experimental hemorrhagic anemia	Hct.	19.0	1st	19	Ferric chloride	10
	Hct.		18.7	2nd	18†	Ferrous chloride	24	
	Hct.		22.5	3rd	6	Ferric chloride	12	
40-149		Experimental hemorrhagic anemia	Hct.	22.0	1st	34	Ferric am. cit.	9
	Hct.		13.4	2nd	9†	Ferrous chloride	39	
	Hct.		22.0	3rd	9	Ferric chloride	3.3	
	Hct.		27.2	4th	10	Ferric chloride	11	
	Hct.		18.3	5th	10	Ferrous chloride	26	
40-115		Experimental hemorrhagic anemia	Hct.	27.0	1st	19	Ferrous chloride	44
	Hct.		21.6	2nd	18	Ferric chloride	24	
	Hct.		28.4	3rd	9†	Ferrous chloride	60	

\* Given with meal of bacon, 2 eggs, French fried potatoes, peas, tea.

† Iron reduced with SO<sub>2</sub>.

Hct. = hematocrit reading.

that of the corresponding ferric salt. Also there was shown to be no difference in the response to iron reduced to the ferrous form by the addition of cevitamic acid as contrasted to that treated with sulfur dioxide.

Since under well controlled conditions there did not seem to be a consistently better use of ferrous salts it was decided to study several subjects who could be relied upon not to take any iron other than that given for tracer studies. Accordingly two young white women whose intelligence and dependability were unquestionable were studied (P. C. and R. H. fig. 1, table 1). In both instances ferrous salts were better utilized.

The last three cases (E. N., E. M. and M. K., fig. 1, group III and table 1) were medical out-patients with typical pictures of iron deficiency anemia. During the course of study they received no therapeutic iron. In each case the ferrous salts were handled with greater efficiency than were the ferric salts.

**DISCUSSION.** By determining the changes in level of serum iron following the administration of ferric and ferrous salts Heilmeyer and Plotner (10) and Moore and his associates (13) have indicated that the latter salts are absorbed to a greater degree. This method has decided limitation however since it assumes fixed relationships between the rate of absorption and rate of removal from the serum, as we have pointed out earlier (5).

Some reported balance studies have tended to give false information concerning the uptake of iron salts. Quite possibly this is due to the methods employed. The determination of iron in the presence of large quantities of calcium salts and phosphates has repeatedly been shown to be extremely difficult; this has been stressed in an earlier communication (9). Most iron balance studies seem to err in the direction of positive balances. This might be explained by the systematic loss of iron in ashing processes or in the incomplete determination of iron in the excreta due to interfering calcium and phosphorus compounds. Recently, March et al. (11) reported studies of a group of normal young women whose iron absorption was estimated by balance methods. They found as much as 50 to 60 per cent of administered iron (dose 30-50 mgm.) was absorbed. They also claimed that this sort of positive balance existed with normal daily intakes. It can be seen that should this rate of absorption continue, these women would at the age of fifty years have retained iron in amounts found only in hemachromatosis, since the capacity of the body to excrete iron is known to be nearly negligible. Such markedly positive balances probably occur only for short periods of time when the body reserves of iron have been depleted. As recently pointed out by McCance and Widdowson, iron balance studies are generally useless (12).

#### SUMMARY

In a series of nine human subjects to whom iron tagged with radioactive isotope was administered it appeared that the ferrous iron salt is much more readily absorbed and subsequently utilized than the corresponding ferric salt or ferric ammonium citrate fed under the same conditions.

In eleven experiments conducted on three dogs in which an iron deficiency anemia of long duration had been established and maintained by bleeding, there was a considerably greater uptake of ferrous than of ferric iron.

Individual response to a given dose of tagged iron is so variable that it is not feasible to study the relative utilization of different iron preparations in different subjects. It is necessary that studies be controlled in the same individuals to eliminate this source of error.

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# HEMORRHAGIC SHOCK: A METHOD FOR ITS PRODUCTION AND A FORMULA FOR PROGNOSIS<sup>1</sup>

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Our previous method of graded hemorrhage and transfusion was developed mainly in order to test the value of different plasma substitutes (3-5, 9, 11). The differentiation between blood substitutes and plasma substitutes is important. There is no known blood substitute, but a number of plasma substitutes are being used or considered. One disadvantage of the previous method of producing shock by graded hemorrhage for assaying a plasma substitute was that a high degree of anemia was produced by repeated bleeding. It is apparent that, while a plasma substitute may overcome the effects of oligemia and plasma loss, it cannot overcome those of profound anemia. This was apparent in some of our early experiments in which hematocrit and hemoglobin fell to very low levels following the repeated hemorrhage. Since our studies were directed at an evaluation of plasma substitutes, it seemed more logical to prevent a profound anemia which might affect the results adversely. Reinfusion of the red cells removed at the bleeding was added to our experimental procedure. The assay of the test material thus became a matter of substituting the test material for the plasma removed at the hemorrhage. As will be seen later, some red cell loss was unavoidable, because part of the red cells from each hemorrhage had to be retained for various analyses, but no serious anemia was ever produced.

**METHODS.** Unanesthetized dogs were prepared under local procaine anesthesia. One carotid artery was used for the continuous recording of blood pressure with a glass capsule or mercury manometer. Heparin was used as an anticoagulant. One femoral artery was cannulated for the withdrawal of blood and one femoral vein for the infusion. The other femoral vein was exposed for direct injection of Evans' blue for the determination of plasma volume. The following determinations<sup>2</sup> were carried out at intervals; hemoglobin, hematocrit, sedimentation rate, total plasma proteins, albumin and globulin, arterial plasma CO<sub>2</sub> content and plasma volume. Blood volume was calculated from plasma volume in the usual way. Hematocrit was corrected for the residual plasma. Chemical determinations were carried out on arterial blood. The dog was restrained only as much as necessary. Small amounts of procaine were applied

<sup>1</sup> A preliminary report was published in the Federation Proceedings (1) and by the National Research Council (2).

This work was done under the auspices of the Committee on Research in Shock, of the Michael Reese Hospital, and was supported by the Michael Reese Research Foundation, and a grant from the Edible Gelatin Manufacturers' Research Society of America, Inc.

<sup>2</sup> For the chemical methods used, see (9).

to the incisions when required. Twenty-five, 23, 21, 19 and 17 per cent of the original measured blood volume was withdrawn at hourly intervals. One per cent of the original blood volume was retained at each hemorrhage and one per cent was retained at each infusion for determinations, and not returned to the animal. Thus a total of 10 per cent of the original blood volume was withheld. The rest of the blood (i.e., 23, 21, 19, 17 and 15 per cent of the original blood volume) was returned to the animal  $\frac{1}{2}$  hour after each hemorrhage. The blood was centrifuged and the plasma and packed cells were infused separately. This was done in order to simulate conditions in experiments with infusion of saline or of plasma substitutes, but it was found that this part of the method was not essential.

*Probability of survival.* Despite attempts to standardize the conditions for producing shock by hemorrhage, the individual variability of the dog is so great that the evaluation of any shock combatting agent is difficult and inaccurate. Others and we have tried to overcome this variability by taking into account individual determinations in the animal such as blood pressure, plasma CO<sub>2</sub>, hematocrit, blood potassium, bleeding volume, bleeding time, blood volume, etc. (3-5); others have refused to accept any of the above criteria and have relied on the time of terminal issue and on typical autopsy findings. None of the above methods allows for a simple evaluation of the condition of the experimental animal before death and autopsy (6-8).

From a review of the available methods and from our own experience, we concluded that it would be most desirable to know at the beginning of the experiment which animal would live and which would die with or without therapy. If we could account at the beginning of an experiment for the variability of the animal, we thought we might more quickly and more accurately assay a therapeutic agent.

We have, in the past, attempted to evaluate the animals' resistance to shock by many of the above means. Like others, we found that blood pressure was in general unreliable as an early indication of impending shock, or even of shock. Of all the determinations we have found that the plasma CO<sub>2</sub> content showed the closest correlation to the subsequent course of the experiment (3-5). This is to be expected, for plasma CO<sub>2</sub> is probably an expression of a number of vital factors in the condition of the animal in impending shock or in shock, such as circulation, state of hypoxia, tissue oxygenation, acidosis, and organ function, particularly of liver and kidney.

The product of the arterial plasma CO<sub>2</sub> content in volumes percent and of the maximal blood pressure in millimeters of mercury, both taken thirty minutes after the first hemorrhage, appeared to correlate even better with the length of survival of the animals. We have called the above product the "Factor of Probability of Survival" (F. P. S.).

**RESULTS.** The data on CO<sub>2</sub>, blood pressure, F. P. S. and survival time of 41 dogs presented in table 1, have been subjected to statistical analysis and the following results were obtained.<sup>3</sup>

<sup>3</sup> We are indebted to Mr. Herbert Silverstone from the Department of Cancer Research of Michael Reese Hospital for the analysis.

TABLE 1

*Probability of survival of dogs subjected to repeated graded hemorrhage, hypotension and reinfusion with their own blood*

(Arranged according to arterial plasma CO<sub>2</sub> content and factor of probability of survival, 30 minutes after first hemorrhage)

B.P. MM. Hg	FACTOR OF PROBABILITY OF SURVIVAL	HRS. OF SURVIVAL FROM BEGINNING OF EXPERIMENT	B.P. MM. Hg	FACTOR OF PROBABILITY OF SURVIVAL	HRS. OF SURVIVAL FROM BEGINNING OF EXPERIMENT
30 minutes after 1st hemorrhage			30 minutes after 1st hemorrhage		
CO <sub>2</sub> 11.3-14.9 vol. %			CO <sub>2</sub> 25-29.9 vol. %		
56	767	2	46	1288	3
97	1280	2	58	1438	13
94	1307	4	61	1708	48
125	1413	3	80	2096	3
			75	2138	13
			82	2312	48
			84	2335	48
			102	2550	13
			112	3069	48
CO <sub>2</sub> 15-19.9 vol. %			CO <sub>2</sub> 30-34.9 vol. %		
48	859	2	78	2293	48
66	1069	2	74	2300	13
74	1184	3	68	2312	4
80	1312	1.5	81	2770	13
98	1460	1	104	3224	48
90	1719	3	120	4116	48
CO <sub>2</sub> 20-24.9 vol. %			CO <sub>2</sub> 35-39.9 (one 42.6) vol. %		
42	865	2	44	1712	48
50	1130	3	63	2180	13
56	1148	48	72	2750	48
82	1599	13	77	2834	48
70	1631	4.5	80	3088	48
78	1708	4	105	4473	48
85	1972	48			
102	2009	48			
97	2018	13			
92	2107	2.5			

TABLE 2

*Analysis of the average values of table 1*

CO <sub>2</sub>	NO. OF DOGS	B.P. MM. Hg	FACTOR OF PROBABILITY OF SURVIVAL	HOURS SURVIVAL	PER CENT OF ANIMALS		
					Died during expt.	Died during night	Surviv. indefinitely
Vol. %							
11.3-14.9	4	93	1192	2.8	100	0	0
15.0-19.9	6	76	1267	2.1	100	0	0
20.0-24.9	10	75	1618	18.6	50	20	30
25.0-29.9	9	78	2104	26	22	33	45
30.0-34.9	6	87	2836	29	17	33	50
35.0-39.9	6	74	2840	42	0	17	83
(one 42.6)							

Blood pressures (taken 30 min. after the first hemorrhage) and survival times showed a poor correlation. This is evident for the case of the actual pressures from simple inspection of the data of table 1 and the summary in table 2. Eight experimental animals with a maximal blood pressure of 80 mm. Hg or more failed to survive the experiment, while three other animals with blood pressures of 40 to 60 mm. did survive the experiment. There was some correlation between actual blood pressure and survival when the 41 experiments were divided into 2 groups; this correlation was improved slightly, when blood pressures were expressed as percent of the control values.<sup>4</sup> Yet, the degree of correlation was so small that it was evident that blood pressures (actual or in percent of the controls) taken 30 minutes after the first hemorrhage, could be of value in predicting the ultimate survival of a number of animals in very large series of experiments only.

CO<sub>2</sub> values and survival times gave an excellent correlation insofar as there was an unquestionable upward trend in probability of survival with increasing CO<sub>2</sub> values. This is shown clearly in tables 1 and 2. No animal with a CO<sub>2</sub> below 20 survived the experiment. Above 20 vol. percent there was a practically arithmetic increase of survival time. When the animals were grouped according to CO<sub>2</sub> values, the following data were calculated with the chi square test:  $X^2 = 9.44$ ,  $df = 2$ , and  $P < 0.01$  (Fisher). There is therefore a statistically significant correlation between the CO<sub>2</sub> and the probability of survival. The F. P. S. showed as comparable a correlation with survival time as did the CO<sub>2</sub>. This is somewhat surprising, since blood pressure had a wide spread in scattergrams and had a poor correlation with survival times. This, we believe, can be explained to a great part by our observation that, in a number of cases, the F. P. S. predicted the ultimate outcome of the complete experiment (5 hemorrhages) better than the CO<sub>2</sub> value; i.e., in animals with relatively good CO<sub>2</sub> values but with poor blood pressures, the F. P. S. was low, indicating a poor chance for survival. The F. P. S. seems to be about 10 per cent better in predicting the outcome of an experiment than the CO<sub>2</sub> value. This difference is not apparent in the statistical treatment of the data. Yet, on biological grounds, we feel that in some instances in the presence of a good blood pressure, despite a relatively low CO<sub>2</sub>, the animals recover. For that reason we are continuing to use the F. P. S. as a means to predict the chance of survival of a dog following 5 graded hemorrhages. The data on table 3, arranged according to the F. P. S., illustrate the preceding statement.

It is seen that with a F. P. S. below 1,700, only one out of 16 dogs survived more than 24 hours, whereas with a F. P. S. above 3000, all 5 animals lived more than 24 hours. The range of the F. P. S. between 1,700 and 2,300 or 1,700 and 3,000 seems to be the most favorable zone for the assay of plasma substitutes.

Table 4 presents the results of 10 experiments on repeated hemorrhage and reinfusion of 0.9 per cent saline solution and the dog's own red cells. None of the

<sup>4</sup> A high degree of correlation between the actual and the per cent of the initial blood pressures at 30 minutes after the first hemorrhage was found. Some animals had rather high control blood pressure.

animals survived the experiment regardless of high values for  $\text{CO}_2$  and the F. P. S., although there was evidence of greater resistance when  $\text{CO}_2$  values and the F. P. S. were higher. It is worth noting that only dogs with good  $\text{CO}_2$  values and a high F. P. S. were used in this group.

DISCUSSION. The results presented demonstrate several facts.

1. Blood pressures, 30 minutes after a hemorrhage of 25 per cent of the total measured blood volume, are a poor index of the condition of an animal and of the prognosis.

TABLE 3

*Summary of data of table 1 showing relation between factor and periods of survival*

SURVIVAL	FACTOR OF PROBABILITY OF SURVIVAL			
	Below 1,700	1,700-2,300	1,700-3,000	Above 3,000
1 Died before fifth infusion (4½ hrs.).	13	4	5	0
2 Died during night (13 hrs.).....	2	3	6	0
1+2 Survived less than 24 hours....	15	7	11	0
3 Survived +24 hours.....	1	5	9	5

TABLE 4

*Factor of probability of survival in dogs subjected to repeated graded hemorrhage, hypotension and infusion with saline solution plus their own red blood cells*

EXP. NO.	DOG		BLOOD VOLUME CONTROL (EVANS BLUE)	CO <sub>2</sub> (ARTERIAL PLASMA)		BLOOD PRESSURE		FACTOR OF PROBABILITY OF SURVIVAL*	PERIOD OF SURVIVAL
	Sex M. F.	Wt.		Control	30 minutes after first hemorrhage	Control	30 minutes after first hemorrhage		
		kgm.	cc.	Vol. %		mm. Hg			
1	M	13.2	1490	50.0	28.5	190	68	1938	Hemorrhage 3
2	M	10.8	963	44.1	23.3	130	92	2143	Hemorrhage 3
3	F	12.0	1274	46.6	22.9	184	105	2404	Hemorrhage 3
4	M	11.4	1356	47.9	27.7	128	90	2493	Hemorrhage 4
5	M	11.6	1190	39.5	27.7	164	105	2909	Hemorrhage 4
6	M	8.2	975	35.4	26.1	188	115	3002	Hemorrhage 4
7	M	8.3	771	51.2	39.7	160	86	3404	Hemorrhage 3
8	F	14.2	1545	45.0	36.9	208	114	4207	Hemorrhage 5
9	F	15.4	1920	47.0	35.4	174	147	5204	Hemorrhage 3
10	M	7.6	820	46.5	42.0	166	130	5460	Hemorrhage 4

\* Blood pressure  $\times$   $\text{CO}_2$ , 30 minutes after hemorrhage 1.

2. The arterial plasma  $\text{CO}_2$  content taken at the same time is a reliable index of the condition of the greater number of animals and of the prognosis.

3. The product of both values, i.e., of the average maximal blood pressure and the arterial  $\text{CO}_2$  content after the first hemorrhage of 25 per cent of the blood volume, correlates as closely with the subsequent survival of animals subjected to repeated graded hemorrhages and reinfusion of blood as does the  $\text{CO}_2$ . This product of blood pressure and  $\text{CO}_2$  has been named the "Factor of Probability of Survival" (F. P. S.). The F. P. S. seems to have an advantage over

the CO<sub>2</sub> values alone in a number of cases, and its use is being continued, although both values were found to have the same statistical significance in relation to survival time,

4. With reinfusion of blood after 5 graded hemorrhages and 5 periods of thirty minutes each of relative hypotension and hypoxia, animals will survive only when their CO<sub>2</sub> values are above 20 volumes percent, or when their F. P. S. is above 1,700 (one exception for the F. P. S. in 16 tests). On the other hand, all animals with a F. P. S. above 3,000 survived.

5. In similar experiments where 0.9 per cent saline solution replaced the plasma and where most of the red cells were returned to the animal, none of the 10 dogs survived the experiment. This occurred even though the F. P. S. and the CO<sub>2</sub> values in most dogs were relatively high.

The initial bleeding of 25 per cent of the determined blood volume and the succeeding half hour oligemic period constitute a "provocative test." In an animal with good reserves and good resistance there will be little effect from such a procedure (e.g., expt. 9, table 4). On the other hand, in an animal with poor reserves and low resistance, this procedure may cause definite shock (e.g., expt. 1, table 1). Approximately half of the animals in tables 1 and 3 with a good F. P. S. (1,700–3,000) and all animals with a F. P. S. above 3,000 may not have been in shock or even in impending shock at any time of the experiment (e.g., in the third dog of the group with CO<sub>2</sub> 25–29.9 in table 1). It must be stressed however, that the ultimate survival of animals even with the best values for CO<sub>2</sub> or F. P. S. depends on the nature and effectiveness of the reinfusion fluid.

We conclude therefore that we have in the "provocative test" and in the CO<sub>2</sub> values and the F. P. S. a means of predicting early in the experiment whether an animal is likely to develop shock, and what its chances of survival will be if whole blood is the infusion fluid. With this established as a base, we can then evaluate a plasma substitute of unknown efficacy. The CO<sub>2</sub> or F. P. S. values also tell us whether an animal will be useful for a given experiment, for if the CO<sub>2</sub> or the F. P. S. are too low, no known procedure will save the animal. We have used the F. P. S. in the evaluation of gelatin as a plasma substitute and our experience has been gratifying (2, 10). We are also able to classify our experiments 30 minutes after the first hemorrhage and then choose the infusion fluid or other therapy. We thus do not have to reject experiments after they are completed and after a postmortem has shown absence of typical findings (7) or if other aberrations have become apparent (8). We believe that a series of 10 dogs with a CO<sub>2</sub> between 25 and 35 volumes per cent or with a F. P. S. of between 1,700 and 3,000 can yield adequate information about the value of a plasma substitute.

The CO<sub>2</sub> or the F. P. S. provide a measure of the individual differences in animals or in groups of animals, due to varying degrees of hydration, disease, sex, age, resistance to trauma or hemorrhage and to seasonal effects. The latter factor is of interest, because we have encountered certain weeks or months when a large proportion of dogs had either low or high CO<sub>2</sub> values or factors, which seemed to depend partly on the weather and on the degree of hydration of the animals. By classifying and grouping experiments, so that animals of similar levels of shock resistance are compared, we can more accurately evaluate a plasma

substitute or other therapy. Unless some method such as this is employed, it is almost impossible to compare two different therapeutic agents, unless the difference in value of the two agents is very striking (such as plasma and saline), or unless large numbers of animals are used in order to overcome the error due to individual variations. We realize that the  $\text{CO}_2$  value or the F. P. S. is not a highly accurate means of ascertaining the animals' reserves and resistance to shock. Perhaps by including some other values such as thiocyanate space, etc., we might improve our means of predicting the resistance of given animal to graded hemorrhage.

We have also found that it is possible in the dog to improve the F. P. S. before the experiment and thus to improve the resistance of the animal to blood loss, surgical manipulation, etc.

It is hoped that a safe "provocative test," and a formula comparable to the F. P. S. can be developed for man.

The value of saline in the treatment of hemorrhagic shock has been denied by many (4, 6, 11) and supported by a few workers (12 and others). Warren et al. (13) have shown that infusion with saline solution in amounts leading to edema may prolong the life of such animals. We believe that saline has little value in the treatment of shock from hemorrhage in dogs.

#### SUMMARY AND CONCLUSIONS

Hemorrhagic shock could be produced in dogs by 5 hourly hemorrhages, withdrawing 25, 23, 21, 19 and 17 per cent respectively of the original blood volume; after a half-hour period of relative hypotension and hypoxia, each bleeding was followed by reinfusion of respectively 23, 21, 19, 17 and 15 per cent of the withdrawn heparinized blood.

The level of the blood pressure  $\frac{1}{2}$  hour after the first bleeding was of little value as an index of the actual condition of the animal or the prognosis as to survival.

The level of arterial plasma  $\text{CO}_2$  content  $\frac{1}{2}$  hour after the first bleeding was found to parallel roughly the length of survival of the dogs.

The product of the maximal blood pressure and of the arterial plasma  $\text{CO}_2$  content 30 minutes after withdrawal of 25 per cent of the original blood gives a value which parallels the length of survival of dogs subjected to repeated graded hemorrhages and reinfusion of blood as good as or possibly better than the  $\text{CO}_2$  values. This factor has been named the "Factor of Probability of Survival" (F. P. S.).

Dogs with a low F. P. S. (below 1,000) or with a low  $\text{CO}_2$  value (10–20 vol. per cent) did not survive the graded hemorrhage alternating with reinfusion of blood. At a F. P. S. between 1,700 and 3,000, or at  $\text{CO}_2$  values between 20 and 30 volumes per cent, about half of the animals survived more than 24 hours. With a F. P. S. above 3,000 all animals survived indefinitely.

Infusion with saline 0.9 per cent, plus the animal's red cells (i.e., replacing the plasma with saline) failed to save any of the dogs beyond the duration of the experiment, in spite of very high factors (3,000–5,460) and relatively high  $\text{CO}_2$  values (in 7 out of 10 dogs).

The CO<sub>2</sub> and the F. P. S. give us the means to predict the probable course of the experiment and the value of a plasma substitute, and to classify individual animals according to their resistance to shock.

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# RESIDUAL DISTURBANCES IN THE HIGHER FUNCTIONS OF THE C. N. S. INDUCED BY OXYGEN AT HIGH PRESSURE<sup>1</sup>

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The reactions of animals exposed to oxygen at high pressure leave little doubt that the central nervous system is profoundly affected under such conditions, especially when the O<sub>2</sub> pressure employed is above three atmospheres.

In addition to convulsive attacks, numerous lesser manifestations of nervous involvement occur such as hyperexcitability, disturbances in equilibrium, hypotonicity, states of stupor and coma. Most animals possess a striking capacity for recovery but such recovery is rarely immediate on return to the normal environment so that in the post-decompressional period very obvious abnormal states persist for periods of a few minutes or hours up to several days, depending largely upon the severity of the exposure and the susceptibility of the animal. Occasionally after an initial but partial recovery various types and degrees of dysfunction are retained permanently, among these the most notable are spastic paralyses suggestive of C. N. S. damage. These post-decompressional manifestations of C. N. S. involvement can be augmented by short exposures to high O<sub>2</sub> pressure repeated at appropriate intervals (Bean and Siegfried, 1943) which indicates that these residual effects have a cumulative influence.

These results suggest that perhaps exposures to O<sub>2</sub> at high pressure which were insufficient to induce any overt residual effects might still alter some of the higher C. N. S. functions. Experiments were therefore carried out to determine whether this does occur.

**METHOD.** The experimental animals chosen for this study were young albino rats. The C. N. S. functions selected for investigation were those of learning and memory. The Lashley Maze III (Lashley, 1929) was employed to test these functions.

The exposures to the increased O<sub>2</sub> pressure took place in a pressure chamber provided with a device for continuously circulating the O<sub>2</sub>, and particular care was taken to eliminate the possibility of an accumulation of even very small amounts of CO<sub>2</sub> within the chamber; to this end soda lime was spread over a large area of the chamber not accessible to the animals, in addition to the CO<sub>2</sub> absorbent contained within the cartridge of the ventilating device.

In order to ensure high concentration of the O<sub>2</sub> and to eliminate any possible danger of nitrogen bubble formation on decompression, pure O<sub>2</sub> (U.S.P.) was used and the chamber thoroughly washed free of air by a rapid flow of O<sub>2</sub> from the supply tanks before the chamber pressure was raised. The chamber tem-

<sup>1</sup> Preliminary Report in Proc. Soc. Exper. Biol. and Med. 54: 134, 1943.

perature was held at approximately 25°C. The O<sub>2</sub> pressure employed was for the most part slightly in excess of five atmospheres absolute.

The use of the maze for testing the animals made it imperative that the exposures to O<sub>2</sub> at high pressure be so regulated as to avoid the induction either of any motor disability which might interfere with the animals' locomotion or any other adverse effect such as pronounced pulmonary damage or gastrointestinal disturbance which might alter the animals' motivation in the maze. The duration of the exposures was therefore short. Based on the observations in earlier experiments the upper limit of exposure time was arbitrarily set at 15 minutes, but because some of the animals were convulsed even in less than this, the duration of some exposures was less than 15 minutes. The regulation of the exposure time therefore necessitated the constant observation of the animals by the operator, stationed outside the chamber. In spite of these precautions to prevent convulsive seizures or other overt manifestations of O<sub>2</sub> poisoning some seizures did occur due to the unpredictable reactions of some animals, especially in the first few of a series of successive exposures. Although convulsive attacks might be avoided in the period of maintained high pressure, they were peculiarly prone to occur during decompression, which introduced a complication in the prevention of such attacks. In order to eliminate the possibility of O<sub>2</sub> emboli, decompression was carried out in stages over a period of time about equivalent to the stay at the increased pressure.

Except in the preliminary exploratory experiments, the animal to be tested was subjected to a series of 16 exposures made over a period of 9 days, but with no more than 2 exposures per day. One day of rest followed the last of each series of 16 exposures before the animal was subjected to its test in the maze. Thus 10 days elapsed between beginning the O<sub>2</sub> exposures and beginning the maze run. This interval of one day's rest between the last exposure to O<sub>2</sub> and the maze test was necessitated by the finding in the first few preliminary experiments that although animals may have shown no convulsive seizures or obvious motor disabilities while under the increased O<sub>2</sub> pressure, they commonly presented the appearance of being mentally confused and dazed during decompression or after their return to their normal environment and when placed in the maze refused to run for several hours or half a day.

*Procedure in the determination of the effects of high O<sub>2</sub> pressure on learning.* In the learning tests 31 young adult rats selected from the stock of the Psychology Laboratory were divided into two groups; one, of 16 animals to be used as the test group (A) and the other of 15 animals to be used as the control group (B). Both groups were treated in the same way throughout except that the animals of the test group were subjected to a series of 16 exposures to O<sub>2</sub> at high pressure before learning the maze.

The criterion of an animal's having learned the maze was arbitrarily chosen as 10 errorless runs in 10 trials in the maze, on each of two successive days. The indices of facility in learning selected were: (1) the minimum number of trials necessary to attain the designated criterion of having learned the maze, and (2) the number of errors made in that learning.

The learning procedure used for both the test and control groups was as follows: The animals were fed in the food box of the maze for 10 successive days to accustom them to handling, a 24 hour feeding schedule, and to establish the food association. Maze learning was then begun by giving each animal one trial in the maze on the first day of the learning period, 2 trials on the second day, 3 trials on the third day, 4 on the fourth day, and 10 trials on each succeeding day until the designated learning criterion was reached.

Test group procedure: The animals in the test group (A) after having had 10 days of feeding in the maze food box, were subjected to the increased O<sub>2</sub> pressure in a series of 16 exposures over a period of 9 days as mentioned above, avoiding so far as possible convulsive seizures. After resting in their home cages on the 10th day they were started learning on the 11th. The maze learning procedure was the same as that described above. The number of trials and errors made in reaching the criterion was recorded for each animal. The procedure for the control group (B) was identical except that they rested while the test group (A) was exposed to the increased O<sub>2</sub> pressure.

*Procedure for determining the effects of high O<sub>2</sub> pressure on memory and retention.* In the memory and retention tests those animals which had served as the controls (B) in the learning experiment and which had therefore already learned the maze were subdivided into two groups: Control group (C) made up of 7 animals, and test group (D) of 7 animals. (One of the original 15 animals in the control group (B) for learning was accidentally lost.) After having ascertained that both the control and test groups (C and D) had again reached the designated criterion following the learning test, each animal of the test group (D) was subjected to a series of 16 exposures to the increased O<sub>2</sub> pressure in the same manner as employed in the learning tests, and after the interval of one day rest was subjected to the maze on the 11th day to determine retention of the maze habit. The total number of trials and errors made in again reaching the criterion was recorded. Each of the animals of the control group (C) was similarly treated except for the omission of the exposures to increased O<sub>2</sub> pressure.

In order to supplement the data on retention and memory provided by groups C and D, other rats, which had previously learned the maze in another connection but which had not been exposed to increased O<sub>2</sub> pressure, were employed. These animals were brought up to the designated criterion of maze mastery and then were divided into two groups, control group E, of 8 animals, and test group F, of 9 animals.

Each of the animals of the test group (F) was subjected to a series of 16 exposures to increased O<sub>2</sub> pressure just as had been those of test group (D), and following the customary day of rest was tested for retention of the maze habit. The control group (E) was similarly treated except for the omission of the exposures to the increased O<sub>2</sub> pressure.

RESULTS. 1. *The effects of successive exposure to O<sub>2</sub> at high pressure on learning.* The data on the total number of errors and trials made in learning the maze by the control group of animals (B) and the test group of animals (A) are shown in tables 1 and 2.

These data show that exposure to O<sub>2</sub> at high pressure as carried out in our experiments had no significant effect on learning as judged by two indices: (1) total errors performed in reaching the criterion and (2) the total trials needed to reach the criterion.

2. *Effects of exposure to O<sub>2</sub> at high pressure on retention and memory.* In contrast to the absence of any significant effect of exposure to O<sub>2</sub> at high pressure on learning, the effects of such exposure on memory and retention were quite pronounced as is shown in the data summarized in tables 3 and 4.

In the first test group (D) the mean number of trials to regain the criterion of 2 errorless days on 2 successive days following the exposure increased O<sub>2</sub> pressure

TABLE 1

*The effects of exposure to high O<sub>2</sub> pressure on learning as judged by the number of errors made in reaching the criterion*

GROUP	NUMBER OF ANIMALS	NO. OF ERRORS TO REACH THE CRITERION OF LEARNING	MEAN DIFF.	DEGREES OF FREEDOM	FISHER'S t	PROBABILITY OF OCCURRENCE BY CHANCE
						%
Test (A).....	16	51.2	2.3	29	0.154	88
Control (B).....	15	48.9				

TABLE 2

*The effects of exposure to high O<sub>2</sub> pressure on learning as judged by the number of trials made in reaching the criterion*

GROUP	NUMBER OF ANIMALS	NO. OF TRIALS TO REACH THE CRITERION OF LEARNING	MEAN DIFF.	DEGREES OF FREEDOM	FISHER'S t	PROBABILITY OF OCCURRENCE BY CHANCE
						%
Test (A).....	16	51.9	-5.4*	29	0.563	58
Control (B).....	15	57.3				

\* The trend is the opposite of that found for errors, i.e., the test group took fewer trials to reach the criterion hence is represented by (-)5.4.

was 75.7 whereas in the corresponding control group (C) it was only 15.7. A similar large difference was found in case of the errors made in relearning; the mean number of errors for the test group (D) was 58.7 whereas in the control group (C) it was 5.0. Equally striking differences were found in groups E and F. The mean number of trials taken to relearn the maze for the test group (F) was 57.8 as compared with 6.3 for the control group (E), and the mean number of errors made by the test group (F) was 48.3 as compared with a mean 0.6 error for the control group (E). These four differences are significant since they could occur less than one per cent of the time by chance.

Although the test groups required a significantly greater number of trials

and made more errors than the control groups in relearning the maze, all animals of the test groups finally reacquired the habit. The number of errors and trials taken by the test groups for relearning was not significantly different from the number taken for the original learning. These facts may be considered as further

TABLE 3

*The effects of exposure to high O<sub>2</sub> pressure on retention and memory as shown by the number of trials and errors made in re-attaining the criterion*

RAT	CONTROL GROUP C		RAT	TEST GROUP D	
	No. of trials	No. of errors		No. of trials	No. of errors
118 F	90	27	165 F	80	130
213 F	20	8	117 F	70	106
166 M	0	0	163 F	100	75
214 M	0	0	210 M	100	41
212 F	0	0	223 F	100	32
217 M	0	0	222 F	40	21
207 F	0	0	215 M	40	6
CONTROL GROUP F			TEST GROUP E		
203 M	20	3	247 F	50	97
191 F	20	1	240 M	100	58
188 F	10	1	182 F	50	54
239 M	0	0	239 F	50	51
172 F	0	0	242 F	60	49
172 M	0	0	243 F	50	35
202 F	0	0	196 M	50	35
193 M	0	0	189 M	70	30
			185 M	40	26

TABLE 4

*The effects on retention and memory as determined by trials and errors made in re-attaining the criterion*

GROUP	NUMBER OF ANIMALS	MEAN		MEAN DIFF.		DEGREES OF FREEDOM		FISHER'S t		PROB. OF OCCURRENCE BY CHANCE	
		Trials	Errors	Trials	Errors	Trials	Errors	Trials	Errors	Trials	Errors
Test D.....	7	75.7	58.7	60.0	53.7	12	12	3.681	3.294	<1	<1
Control C.....	7	15.7	5.0								
Test F.....	9	57.8	48.3	51.5	47.7	15	15	6.959	6.276	<1	<1
Control E.....	8	6.3	0.6								

evidence that learning ability was not impaired by exposure to high pressure O<sub>2</sub>. Apparently, only retention of the previously acquired habit was impaired.

DISCUSSION. The observation that maze trained rats refused to run the maze for some hours after their removal from the increased O<sub>2</sub> pressure is worthy

of note for it indicates that even those exposures not severe enough to induce obvious motor disturbances commonly leave effects which persist for hours after the animal's return to atmospheric pressure. While this temporary failure to react to the maze situation may simply represent an altered motivation, or some residual effect on sensory mechanisms, it nevertheless must be a reflection of a post-decompressional influence of the high  $O_2$  pressure and indicates that recovery from such exposure is not as rapid or as complete in these animals as casual observation might lead one to suppose.

Because of the necessary interval between the last exposure to  $O_2$  and the testing in the maze our data provide no evidence concerning possible disturbances in learning ability during the immediate post-decompressional period. It is therefore unsafe to infer that because there is no significant alteration in learning as revealed by tests carried out several days after decompression there should also be no effect in the immediate post-decompressional period of the acute recovery phase.

Just why the ability to learn remains unaffected by previous exposure to increased  $O_2$  pressure whereas memory and retention are adversely affected as shown by tests made some days after return to normal pressure, remains a matter of speculation; possibly the difference might be explained on an assumption that the increased  $O_2$  pressure has something of a predilection for those parts of the C. N. S. which subserve these higher functions. While our data indicate that the maze habit is adversely affected by exposure to high pressure  $O_2$ , the tests were not carried out over sufficient length of time to justify any conclusion regarding their possible permanency. But the permanent motor dysfunction, largely of C. N. S. origin which can be induced by more severe, intermittent exposure to  $O_2$  at high pressure (Bean and Siegfried, 1943) suggests that in all probability proportionately greater, and perhaps even permanent adverse effects on higher functions of the C. N. S. might also be induced by more severe exposures to  $O_2$  at higher pressures than those used in the experiments herein reported.

The finding that exposure to high  $O_2$  pressure causes loss of memory in experimental animals is of interest in relation to the disturbance in memory experienced by men under some conditions of deep diving and which has been attributed to a peculiar action of the increased nitrogen tension obtaining under those conditions.

The precipitation of convulsive attacks or lesser neuromuscular reactions either during the maintenance of the increased  $O_2$  pressure or during decompression therefrom, in spite of attempts to avoid them, raises the question of whether there might not be some etiological relationship between the occurrence of the convulsions and the disturbance in memory. But an examination of the data reveals no causal relationship.

Our experiments do not provide sufficient data to justify a final conclusion regarding the effects of single exposures to high  $O_2$  pressure on learning or memory but they do suggest that the less severe and short exposures such as we have employed do induce significant changes in the memory function. The fact, however, that repeated exposures result in effects which are demonstrable, indicates

that following each single exposure there must persist some alteration which, while of itself not evident, contributes to the cumulative effects of repeated exposures.

#### SUMMARY AND CONCLUSION

In experiments carried out to determine what effect, if any, exposure to high pressures of  $O_2$  might have on learning and memory, young adult albino rats were exposed to  $O_2$  (U.S.P.) at a pressure slightly over 5 atmospheres (absolute) for periods of from 8 to 15 minutes in series of 16 successive exposures over a period of 9 days and with no more than 2 exposures per day. Precautions were taken to eliminate possible complications from increased  $CO_2$  in the respired gas and from  $O_2$  emboli on decompression. In so far as possible the induction of convulsive seizures or pronounced neuromuscular disturbances were avoided. The chamber temperature was maintained at about  $25^\circ C$ .

Tests for learning and memory were conducted with the Lashley Maze III. The criterion selected for evidence of having learned the maze was ten errorless runs on each of two successive days. An interval of one day was provided between the last  $O_2$  exposure and the animal's subjection to the maze. Control animals were treated in the same way as the test animals except for the omission of the  $O_2$  exposures.

No significant difference in the ability of the control and test rats to learn the maze was found in tests begun one day after the last of a series of exposures. On the other hand the memory and retention of the maze, previously learned, was adversely affected in a striking manner by the exposures to increased  $O_2$  pressure. This was shown by the large number of trials and errors made by the  $O_2$  exposed animals as compared with the frequently perfect scores of the controls, in relearning the maze. This result was interpreted to mean that the higher C. N. S. functions of memory are adversely affected by intermittent exposures to increased  $O_2$  pressure.

Animals which had mastered the maze and which appeared to have completely recovered from an exposure to increased  $O_2$  pressure so far as objective motor reactions were concerned, commonly refused to run the maze for some hours after their return to normal pressure. This was interpreted as an indication that even in the absence of any gross manifestations of dysfunction, residual effects persist for considerable time in the post-decompressional period.

The enhancement of the adverse effects of increased  $O_2$  pressure on memory by successive exposures as made in our experiments, constitutes proof of a cumulative action of effects which, following single short exposures, may not be readily demonstrable.

While the data indicate that these adverse effects on higher C. N. S. function persist for a matter of several weeks any conclusion regarding their permanency calls for further experimentation.

The disturbance in memory is not dependent upon the occurrence of convulsive attacks either during maintenance of the increased  $O_2$  pressure or during decompression therefrom. It was concluded: that successive mild exposures to  $O_2$

at pressures of about 5 atmospheres (absolute) as carried out in our experiments do not significantly affect learning following an interval of one day after the last of the series of exposures; that memory, as revealed by maze tests, is very significantly affected by such exposures, and that O<sub>2</sub> at high pressure can induce adverse effects on higher C. N. S. functions even though it may not induce obvious neuromuscular dysfunction.

The authors are indebted to the Horace Rackham School of Graduate Studies for partial support of this investigation and to Dr. N. R. F. Maier for helpful advice and criticism and the maze facilities of the Psychological Laboratory for the Study of Abnormal Behavior in the Rat.

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# BLOOD PRESSURE STUDIES ON NORMAL AND VITAMIN E DEFICIENT RATS

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The effect of vitamin E deficiency on the blood pressure of experimental animals has not heretofore been reported. Only a few references are found in the literature concerning the effect of any vitamin deficiency on the blood pressure of experimental animals. Uhlmann (1), using dogs and rabbits, made subcutaneous, intravenous and oral administrations of crude vitamin B commercial preparations. He effected a dilatation of peripheral blood vessels, a slower heart beat and a reduced blood pressure. Baldwin, Cook and Nelson (2) reported the effect of vitamin A and vitamin B deficient diets on the blood pressure of rats. These workers noted a "slight though significant subnormal pressure" in the vitamin A deficient rats. In the vitamin B deficient group "the subnormal pressure was even more marked." Since no work has been reported on the blood pressure of rats held for extended periods of time on a vitamin E deficient diet we are submitting these findings.

**EXPERIMENTAL.** The rats used in this study were females of the Long-Evans strain. Since weaning at 21 days of age the experimental animals had been maintained on a vitamin E deficient diet.<sup>1</sup> The normal control animals were held on a commercial rat biscuit diet supplemented with lettuce three times weekly.

The animals consisted of four groups:

Group 1 were female rats held on the vitamin E deficient diet for a period of one year. Their general physical condition was comparable to the normal control groups except they were lighter in weight (225 grams: 261 grams).

Group 2 were 1 year old normal female animals.

Group 3 were female rats held on a vitamin E deficient diet for two years.

These animals exhibited the typical symptoms of prolonged vitamin E deficiency such as marked striated muscle flaccidity and atrophy, ataxic gait, denuded areas etc. (3-6).

<sup>1</sup> Vitamin E deficient diet:	24
Casein (commercial).....	35
Cornstarch (uncooked).....	4
Salts (McCollum no. 185).....	20
Lard.....	2
Cod liver oil.....	10
Brewer's yeast.....	5
Cellulose flour.....	

The diet ingredients (except for the cod liver oil) were mixed together and allowed to stand at room temperature for two weeks. The cod liver oil was added to the rancid diet just before feeding.

Group 4 were 2 year old normal female rats.

The apparatus (fig. 1) used in taking the systolic blood pressure was a modification of the technic of Williams, Harrison and Grollman (7). It consisted of two metal chambers lined by discontinuous rubber membranes. The indicator chamber, A, was 2 cm. in diameter and was lined by a rubber condom that invested the rat's tail. Colored water was introduced between the metal chamber and rubber condom after the rat's tail had been inserted. The volume changes within the chamber were shown by a column of water which rose into a capillary tube (0.5 mm. diameter) attached to the chamber. The other chamber, B, was 4.5 cm. in diameter and was lined by the finger of a thin surgical glove. The latter membrane invested the base of the tail less snugly. It served as the pres-

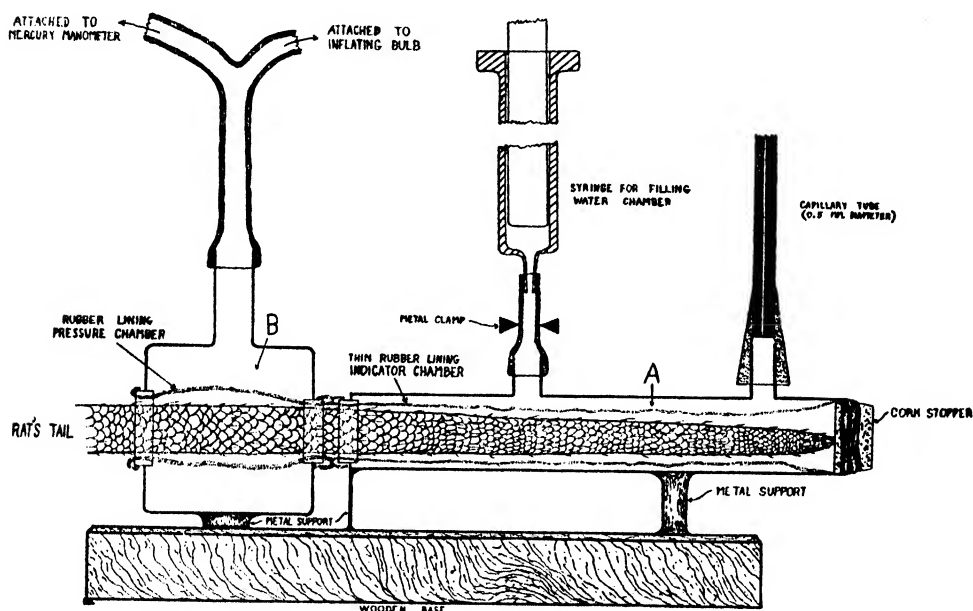


Fig. 1. A sketch of the blood pressure apparatus used in this experiment. A—Water or indicator chamber. B—Air or pressure chamber.

sure cuff to shut off the systolic blood in the tail when air was pumped between the metal chamber and the membrane. A mercury manometer was attached to the air chamber which indicated the pressure (mm. Hg) within the air chamber.

Each rat was anesthetized by an intraperitoneal injection of sodium barbital solution (190 mgm./kgm. body weight). Sodium barbital was selected because of its relatively slight effect on the heart and blood pressure (8). The time interval of narcotization was controlled to prevent any discrepancy due to degree of anesthesia. When the rat was anesthetized, the tail was inserted through the membranes lining the chambers. Water which had been colored with a dye was run into the indicator chamber until it rose into the capillary tube. The pressure cuff was then inflated to a point above the systolic pressure. There was a slight rise in the capillary tube during inflation of the cuff due to venous

congestion in the tail. This had no bearing on the later significant rise. Air was then slowly released from the pressure cuff. When the systolic pressure was reached there was a sudden secondary rise in the capillary tube due to the arterial blood re-entering the tail. At this instant a direct reading was obtained on the mercury manometer. This was the systolic blood pressure of the animal.

We were thus able to obtain series of successive readings. The range of variation of the readings of an animal for any one day was seldom more than 2 to 4

TABLE 1

*Systolic blood pressure in normal and vitamin E deficient rats at room temperature of 35°C.*

GROUP	DIET	AGE	RATS IN GROUP	NUMBER OF MEAS- URE- MENTS	RANGE	MEAN BLOOD PRES- SURE	S.D.*	S.E.†	COMMENTS
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
		yr.		(n)	mm. Hg	mm. Hg			
1	E-deficient	1	6	37	64-86	78.2	6.22	1.02	29.4% reduction in blood pressure due to 1 year of vitamin E deficiency
2	Normal	1	4	24	94-122	110.8	11.94	2.75	
3	E-deficient	2	9	54	48-70	61.7	6.15	0.83	29.2% reduction in blood pressure due to 2 years of vitamin E deficiency
4	Normal	2	7	40	84-94	87.2	2.58	0.41	
1	E-deficient	1	6	37	64-86	78.2	6.22	1.02	21.1% reduction in blood pressure due to 1 year difference in ages
3	E-deficient	2	9	54	48-70	61.7	6.15	0.83	
2	Normal	1	4	24	94-122	110.8	11.97	2.75	21.3% reduction in blood pressure due to 1 year difference in ages
4	Normal	2	7	40	84-94	87.2	2.58	0.41	

\* Of the sample, i.e., of individual measurements.

† i.e., standard deviation of means of samples;  $S.E. = \sqrt{\frac{(x - \bar{x})^2}{(n(n-1))}}$ .

mm. Hg. Each animal was given 4 to 10 readings on each occasion and the mean systolic blood pressure determined.

OBSERVATIONS (table 1). One year old vitamin E deficient rats had an average systolic blood pressure of 78.2 mm. Hg. Normal rats of the same age had an average systolic blood pressure of 110.8 mm. Hg. The deficient animals thus showed a 29.4 per cent reduction in their blood pressure when compared with the control group.

A similar comparison of the two year old animals revealed the deficient animals to have a blood pressure of 61.7 mm. Hg as compared with 87.2 mm. for the

normal rats. This drop is of essentially the same degree of magnitude as that of the blood pressure of the experimental group (29.2 per cent).

In the two E-low groups the older rats showed a reduction of 21.1 per cent in blood pressure when compared with the one year old animals, their respective values being 61.7 and 78.2 mm. Hg.

The normal rats showed an interesting group difference. The two-year old animals had a 21.3 per cent reduction in their systolic blood pressure from that of the year-old group (87.2 mm.:110.8 mm.). This reduction was due to the age differential and not to any dietary difference since all normal animals received the same balanced diet.

**DISCUSSION.** How a deficiency in vitamin E effects a reduction in systolic blood pressure in rats is unknown. A search for the site of the disturbance in the vascular system has not been successful. Histological examination of the hearts of twenty-one deficient animals 12 to 26 months of age, by one of the authors (I. R. T.), failed to show any consistent pathological lesions. Although a few deficient animals showed definite myocardial damage, some of the normal controls also showed myocardial lesions to a similar degree. Thus the lesions were felt to be within normal limits.

A histological examination of the larger vessels, such as the abdominal aorta, iliac, femoral and anterior tibial arteries and their corresponding veins, showed no abnormalities in six 22-month old vitamin E deficient animals.

There are, however, several observations that suggest that vitamin E is essential to the normal functioning of the vascular system. One pertinent point is that the death of the fetuses in vitamin E deficient pregnant female rats has been attributed to any of three conditions. First, a suppression of the development of derivatives in the embryo related to the hematopoietic function, as suggested by Evans and Burr (10) and Uner (11); secondly, defects in the vascular walls causing stasis and extravasation of blood into the tissues, suggested by Mason (12); and last, a combination of both processes.

Another observation suggesting a vascular upset is the presence of edema of the face in young rats suffering from an acute vitamin E deficiency (13). In the case of chicks the vascular disturbances are much more evident and widespread than in the rat. Vitamin E deficient chicks manifest exudative diathesis, widespread edema, cerebellar lesions caused by arterial infarctions, and hemorrhage in the developing fetus (14-16).

The observation that two year old normal rats have a lower systolic blood pressure than year old animals (87.2 mm.:110.8 mm.), and that similar differences were found in E-low rats one and two years of age (61.7 mm.:78.2 mm.), raises a very interesting problem since a rise in blood pressure would be expected in old age and not a reduction. In fact Griffith, Jeffers and Roberts (17) reported "in measuring the blood pressure of hundreds of rats of various ages we have noted a tendency to a slight rise with age." However, rodents rarely develop the deposits of calcium and lipid substances in the media or intima of a vessel (18). Rat studies substantiate this general observation. An explanation for the drop in blood pressure in normal 2-year old animals is, however, still

lacking. The percentage difference is just as large at the end of one year of vitamin E deficiency as after a two year deficiency. The additional drop in blood pressure in the two year old deficient animals was due to an aging effect and not aggravated by the continued deficiency state. The percentage difference between one and two year old deficient rats was 21.1 per cent. The difference between normal rats of corresponding age levels was a 21.3 per cent reduction.

In reporting on blood pressure studies of rats there are a number of factors that materially alter results. Variations in the room temperature have a marked effect upon the blood pressure of rats. In a series of readings nearly as extensive as the data here presented, we noted a consistent lowering of blood pressure at a lower room temperature. Readings taken at a room temperature of 25°C. were approximately 20 to 30 per cent lower than those readings obtained at 35°C. The readings at the higher temperature, however, were probably more accurate because of the greater sensitiveness of our apparatus at the higher temperature. In establishing normal blood pressure of rats it is important to take into account the environmental temperature of the animal. Other authors (7, 19) have recognized this variable and have suggested pre-heating the animal before the experiment in order that the tail may be stimulated to greater thermoregulatory activity. A higher room temperature accomplishes approximately this same purpose resulting in more accurate readings than at a lower temperature.

Normal blood pressure levels in rats cannot be established unless the age of the animals is considered. A survey of the literature shows that this factor has been largely neglected and thus rather wide differences in results have been reported. Our results showed a marked drop in blood pressure in the older group of animals.

The sex of the animals may have some effect on the blood pressure. We compared the blood pressures of six normal 2-year old males and seven females and found the male rats to have a higher blood pressure than the females (101.1 mm.: 87.2 mm.).

#### CONCLUSIONS

1. One year old vitamin E deficient female rats showed a reduction of 29.4 per cent in their systolic blood pressure when compared with normal rats of the same age (78.2 mm.: 110.8 mm.).

2. In two year old vitamin E deficient rats the reduction in blood pressure was essentially the same (29.2 per cent) as in the year-old group. The systolic blood pressure for 2-year old vitamin E deficient rats and normal control rats was 61.7 mm. and 87.2 mm. respectively.

3. A lowering of the blood pressure in the second year of life is reflected equally in both experimental and control groups. This reduction is therefore due to an aging effect and is not a manifestation of a dietary deficiency.

4. An histological examination of the hearts and large vessels of vitamin E deficient rats failed to demonstrate any consistent vascular lesions that might contribute to a reduction of blood pressure.

5. Variations in the room temperature, age and sex of the animal, and methods used in determining blood pressure are variables that influence the blood pressure readings in normal and experimental rats.

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# THE EFFERENT PATHWAY OF CHEMOREFLEX VASOMOTOR REACTIONS ARISING FROM THE CAROTID BODY<sup>1,2</sup>

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The experiments reported here were designed to ascertain which of the several divisions of the efferent vasomotor system actually participate in chemoreflex (carotid body) reactions in the dog and, if more than one, their relative importance and whether they act reciprocally or antagonistically. Such information is requisite to ultimate understanding of the central integration of these reflexes and can be of great value in interpreting the behavior of the chemoreceptors themselves.

The efferent pathway of vasomotor reflexes arising from carotid sinus and aortic pressoreceptors has been studied but the evidence is conflicting as to whether, in the dog, the thoracico-lumbar autonomies provide the sole efferent pathway (Bacq, Brouha and Heymans, 1934; Schneider, 1934; Derom and Grimson, 1939; Dole and Morison, 1940) or whether they act in conjunction with parasympathetic and dorsal root vasodilators (Bayliss, 1902, 1908; Fofanow and Tschalussow, 1913; Tournade and Malmejac, 1932; Bishop, Heinbecker and O'Leary, 1933). Indeed, the whole question of the participation or non-participation of vasomotor fibers other than thoracico-lumbar autonomies in generalized vascular reflexes is confused by unexplained differences in the experimental results of numerous investigators. The present experiments, using a different method and a reflex hitherto unstudied in this connection, contribute to the general problem as well as to the specific question of the mechanism of chemoreflex vasomotor reactions.

**METHODS AND PROCEDURE.** Vasomotor reactions were studied in the submaxillary gland and in the hind leg. These regions were chosen because each possesses, besides the thoracico-lumbar autonomic, a different additional type of vasomotor innervation which might be influenced in its activity by the chemoreceptors.

Change in volume flow of blood provided the index of vasomotor reactions both in the submaxillary gland and in the leg. In the experiments upon the submaxillary gland, the venous outflow was measured, using a drop recorder. Depletion of circulating blood volume which this method usually entails was avoided by continuous automatic reinjection of blood at a rate identical with

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the rate of loss. In the leg, arterial blood flow (femoral artery) was continuously recorded by a modified form of the Gesell and Bronk thermoelectric method (1926).

Since blood flow is not a reliable index of vascular reactions unless the arterial blood is supplied under constant hydraulic conditions, a method of perfusion capable of supplying such conditions was used. This method has been described elsewhere (Bernthal, 1938) but certain of its special features will bear re-emphasis here. The perfusate was normal arterial blood taken directly from the aorta of the experimental animal without exposure to the air and delivered to the perfused part by a "pump" which maintained constant mean and pulse pressures and constant pulse rate while automatically allowing its output to vary in exact accordance with variations in the composite resistance to blood flow (vascular tone) offered by the vascular bed of the tissue being perfused.

Chemoreflex reactions were initiated by injecting chemical agents in solution into the perfusion stream supplying the carotid body region. Conditions were such that the circulation of these agents was confined rigidly to the vascularly isolated carotid bifurcation. Constant hydraulic conditions were maintained in this perfusion circuit as well, in order to obviate variations in stimulation of carotid sinus pressoreceptors. When the submaxillary gland was studied, the ipsilateral Hering's nerve was sectioned and chemical agents administered only to the contralateral carotid body. When vasomotor reactions in the leg were studied, chemical agents were administered to both carotid bodies. The chemical agents used were limited to two, sodium cyanide to stimulate the chemoreceptors and call forth chemoreflex vasoconstriction, and sodium carbonate to inhibit the chemoreceptors and elicit chemoreflex vasodilatation.

The reactions in normally innervated tissue were compared with those in tissue the thoracico-lumbar autonomic innervation of which had been acutely interrupted. In experiments upon the submaxillary gland, the cervical vago-sympathetic trunk was cut leaving the parasympathetic innervation (chorda tympani) as the potential pathway for vascular reactions. In experiments upon the hind leg, unilateral extirpation of the paravertebral ganglionic chain from the thirteenth thoracic to the second sacral segments inclusive left only the dorsal root vasodilator fibers as potential pathways for vascular reactions.

Heparin was injected intravenously and the animals were anesthetized with morphine and urethane or with morphine and chloralose. In most of the experiments, open pneumothorax was instituted and constant artificial pulmonary ventilation maintained in order to avoid changing tensions of the respiratory gases in the blood supplying the vasomotor centers and the tissues in which vascular reactions were to be studied.

**RESULTS AND DISCUSSION.** Chemoreflex vasomotor reactions in the submaxillary gland have not been described heretofore and it was necessary first to establish their presence and nature before modifying the vasomotor nerve supply. Figure 1A demonstrates a typical vasomotor reaction in the submaxillary gland which accompanied exposure of the contralateral carotid chemoreceptors to NaCN. The initial effect was vasoconstriction and this was followed



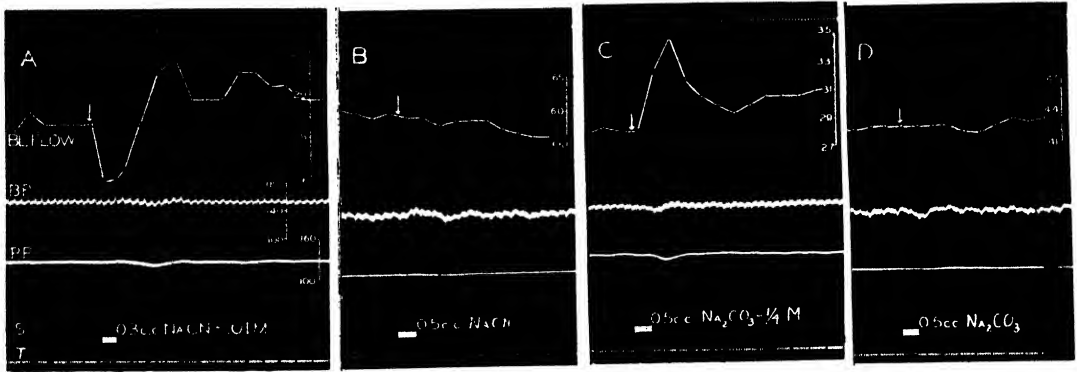


Fig. 1. Chemoreflex vascular reactions in the submaxillary gland and the effects of removing the thoracic-lumbar autonomic impulses.

*Top record*, drops of venous blood leaving submaxillary gland. *BL Flow*, plotted values of blood flow from submaxillary gland in drops per minute. Ordinate scale of linear values for percentile changes in blood flow the same in all records. *BP*, artificially stabilized arterial blood pressure in millimeters of mercury. *PP*, pressure of blood in perfusion circuits supplying submaxillary gland and carotid body; millimeters of mercury. *S*, signal. *T*, time in one and ten second intervals. Record of artificial constant pulmonary ventilation not shown. All records from the same experiment.

A. Effects of sodium cyanide at the carotid body upon blood flow through the submaxillary gland. Innervation of gland intact. B. The same, after section of the cervical vago-sympathetic nerve trunk. C. The effects of sodium carbonate. Innervation intact. D. The same, after section of the vago-sympathetic trunk.

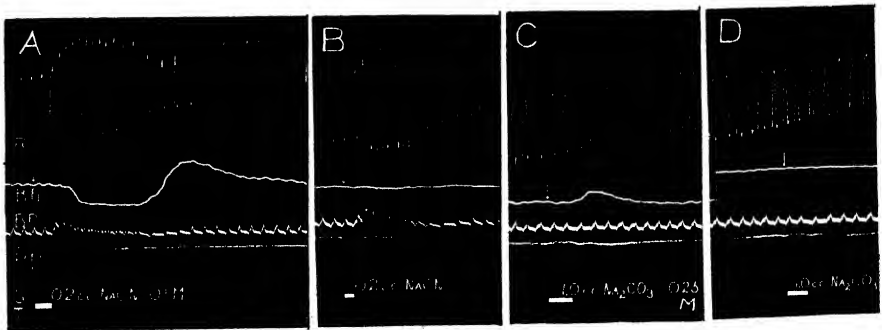


Fig. 2. The effects of sympathectomy upon chemoreflex vascular reactions in the hind leg.

*R*, spirometer record of pulmonary ventilation. *FL*, thermoelectric recording of femoral arterial blood flow. Other abbreviations as in figure 1. Between A and B and again between C and D, the flow recorder was changed from the left (normally innervated) to the right (sympathectomized) femoral artery. All records from the same experiment.

A. Effects of sodium cyanide at the carotid body upon blood flow in the left femoral artery. Innervation intact. B. The same, right femoral artery. Right lumbar and sacral paravertebral sympathetic ganglionic chain extirpated. C. Effects of sodium carbonate at the carotid body upon blood flow in the left femoral artery. D. The same, right femoral artery.

by vasodilatation, varying in degree in different individuals, after which the preadministration level of vascular tone was re-established. The effect of carbonate administration (fig. 1C) was simply vasodilatation followed by recovery. Qualitatively similar reactions to cyanide (fig. 2A) and to carbonate (fig. 2C) can be demonstrated in the hind leg. These observations, considered along with those upon the foreleg (Bernthal, 1938), and upon the intestine (Bernthal and Schwind, to be published) demonstrate a basic similarity in the pattern of vascular reactions occurring simultaneously in widely separated body regions in response to carotid body activity. This fact may have some significance from the point of view of the present inquiry for it suggests dominance of some part of the vasomotor mechanism which is common to all of these regions, specifically the thoracico-lumbar sympathetic.

Whether the thoracico-lumbar sympathetic does in fact dominate the vascular reactions and whether such dominance may be complete or relative is best answered by the parts of the experiment in which this innervation was rendered ineffective (figs. 1B and 1D for the submaxillary gland and 2B and 2D for the hind leg). For most individuals studied, the results are clear cut and definite as these illustrations, taken from typical experiments, indicate. In all experiments upon the leg and in all, with but one and possibly two exceptions, upon the submaxillary gland, there were no recognizable chemoreflex vascular reactions remaining after interruption of the thoracico-lumbar sympathetic pathways. In many of the experiments the flow of blood became absolutely steady following sympathectomy and remained so despite administration of large doses of chemical agents at the carotid body. In a few of the experiments, minor irregularities of blood flow were in evidence after sympathectomy had been performed. However, with the exceptions already noted and to be discussed presently, no relationship could be established between their occurrence and the administration of chemical agents.

Bearing in mind that the endings of the remaining vasomotor pathways were cholinergic, eserine was administered in two of the experiments upon the submaxillary gland with the idea of revealing any reactions which might have been latent or unrecognized in the sympathectomized regions. As might have been expected, upon the basis of established concepts concerning humoral intermediation at autonomic ganglia, chemoreflex vascular reactions in normally innervated tissue were definitely exaggerated following the administration of eserine. But cutting the cervical sympathetic trunk obliterated these exaggerated reactions just as effectively as it did the others. It seems clear, therefore, that in all of these animals (thirteen of a total number of fifteen) the thoracico-lumbar autonomic constituted the sole efferent pathway for the chemoreflex vasomotor reactions observed.

In one experiment upon the submaxillary gland, smaller (15 to 30 per cent of original) but definite and repeatable vasomotor reactions persisted after the cervical vagosympathetic trunk had been severed. In one other experiment small, almost imperceptible variations of blood flow occurred which may con-

ceivably have been chemoreflex reactions. The most obvious explanation, and a definite possibility upon the basis of embryological and neuroanatomical considerations, is the existence in these two animals of aberrant thoracico-lumbar autonomic fibers travelling outside the cervical sympathetic trunk and therefore left uncut. Supporting this interpretation is the precise resemblance of the persisting reactions, both in pattern and in time relations, to those in evidence before nerve section when the thoracico-lumbar innervation was surely in dominance.

Consideration must be given also to the possibility that the chemoreceptors do have central connections with parasympathetic vasomotor neurones. It would seem probable, however, that if such an arrangement does exist it would be present in all individuals of a species and that it could be brought into evidence at least in some degree in all of them. The fact is that only a small fraction of the animals studied displayed reactions which could possibly have been attributed to parasympathetic fibers and, as has been already noted, these responses may equally as well have been mediated over aberrant thoracico-lumbar sympathetic fibers. This consideration, coupled with the fact that none of the experiments on the limb even suggested that dorsal root vasodilators may have been involved, has led us to the belief that in the dog thoracico-lumbar autonomic fibers provide the sole efferent vasomotor pathway for chemoreflex reactions.

*Note.* In view of the lack of agreement in the findings of various investigators concerning the efferent pathway of vascular reflexes initiated at the pressoreceptors, it was a matter of some interest to observe these reflexes under the conditions of our experiments, which we believe are especially well adapted for revealing small reactions. In four of the experiments, adequate observations were made and in no instance did vascular reactions to occlusion of a common carotid artery persist after interruption of the thoracico-lumbar sympathetic pathways. The observations made were all in experiments upon the submaxillary gland and no attempt was made to study pressoreceptor reflexes systematically in various body regions. So far as they go, however, these results support the conclusions of others (*loc. cit.*) that the vascular component of pressoreceptor reflexes is mediated only by the thoracico-lumbar autonomies in the dog.

#### SUMMARY AND CONCLUSIONS

Reflex vasomotor reactions resulting from the action of chemical agents at the carotid body are qualitatively the same in the submaxillary gland as in other body regions in the dog, e.g., sodium cyanide elicits reflex vasoconstriction usually followed by vasodilatation and sodium carbonate elicits simple vasodilatation.

In most individuals (nine of eleven) cutting the cervical (vago) sympathetic trunk completely obliterated chemoreflex vasomotor reactions in the submaxillary gland even though the parasympathetic innervation (chorda tympani) remained intact and even when eserine was administered to potentiate any activity of this innervation. In one and possibly two of eleven animals, small reactions persisted after the vagosympathetic trunk had been severed. It is believed that these reactions were mediated by aberrant fibers of the thoracico-lumbar autonomies.

In all of the four dogs in which it was tried, sympathectomy removed all recognizable chemoreflex vasomotor reactions in the leg even though the dorsal root vasodilator innervation remained intact.

It is concluded that in the dog, certainly in most individuals and probably in all, the thoracico-lumbar autonomic fibers constitute the sole efferent pathway for vascular reflexes originating at the carotid body.

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# EFFECT OF LIVER FRACTIONS ON MITOSIS IN REGENERATING LIVER<sup>1</sup>

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When the median and left lateral lobes of the liver of the rat are removed, the remaining lobes grow rapidly until the original bulk of the liver is restored. Brues and Marble have studied the rate of mitosis in regenerating rat livers by microscopic examination of liver sections (1). By using suspensions of nuclei obtained from liver by the citric acid technique (3) the mitosis rate can be determined by counts made with a hemocytometer. For simplicity only nuclei in metaphase and anaphase were classified as being in mitosis. This provided a rapid assay method which made it possible to carry out the investigations reported here.

**METHODS.** The initial experiments were made with rats of the Slonaker strain which were later discarded because of an epidemic of respiratory infections. Subsequent experiments were performed on rats of the Curtis strain. Both strains have been highly inbred by brother sister matings. All tests were carried out on one month old rats weighing 45 to 60 grams. Operations on the animals were carried out under aseptic conditions.

An incision was made in the midline extending from about  $\frac{1}{4}$  to  $\frac{1}{2}$  inch on either side of the xiphoid process in an anteroposterior direction. The abdominal wall was cut along the midline from  $\frac{1}{4}$  inch posterior to the tip of the xiphoid process which was dissected free of its attached muscle and fascia. With gentle pressure on the sides of the animal, the median and left lateral lobes of the liver were delivered to the surface and their fascial attachments cut. They then were tied at the base with cotton or linen thread and cut as closely as possible to the tie. On a diet of Purina dog chow, survival was 100 per cent unless there was a gross error in the technique, for example, perforation of the diaphragm or cutting of a large blood vessel.

Twenty-four hours after the partial hepatectomy the substance to be tested was injected into the femoral vein and 3 hours later the liver was perfused with saline and removed. In experiments in which  $P^{32}$  was used it also was injected into the femoral vein as  $Na_2HPO_4$  from 5 to 10 minutes after the injection of the test material. In no case did the amount of  $Na_2HPO_4$  exceed 1.5 mgm. Nuclei were isolated and mitotic counts made as previously described (3). Animals were fed Purina dog chow except during a period when diets were prepared in our own laboratory. When fed a diet containing 13 per cent fat (largely sardine and corn oil) the animals were, on the average, 10 grams heavier than when fed the chow,

<sup>1</sup> The work described in this paper was done under a contract recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the University of California.

but did not survive the partial hepatectomy. When the fat content was reduced to about 7 per cent the usual survival rate of 95 per cent was observed. On this diet, however, the mitotic rate one day after operation was reduced to from 0.2 to 0.5 per cent as compared with a rate of from 0.6 to 1.0 per cent, obtained on the dog chow. Except as otherwise indicated, counts of nuclei were made from pooled livers of 5 or more animals. In all experiments controls were run at the same time as the substance to be tested. The animals used as controls received 0.85 per cent saline intravenously except in a few cases in which isotonic  $\text{Na}_2\text{HPO}_4$  containing  $\text{P}^{32}$  was administered. Except as otherwise indicated, all substances were dissolved or suspended in saline solution for intravenous injection.

*Preparation of materials tested.* All preparations, except as mentioned, were carried out in a cold room at a temperature of from 3° to 5° F. Whenever necessary, the salt concentration was adjusted by dialysis or dilutions to 0.9 per cent NaCl. Particulate materials were then centrifuged, resuspended in from 2 to 5 volumes of 0.9 per cent NaCl and passed through a no. 27 hypodermic needle before injections.

*Description of table 1. No. 1-no. 3. Sediment, supernatant fluid and filtrate:* Rat liver was perfused with 0.9 per cent NaCl and blended into 4 volumes of 0.9 per cent NaCl for 2 minutes in the Waring blender, 0.3 M NaOH was added during the blending in amounts sufficient to bring the pH to 7.4. The suspension was centrifuged for 15 minutes at 2,500 r.p.m. Small granules were removed from a portion of the supernatant fluid by filtration by suction with the aid of liberal amounts of Johns-Manville Superceel.

*No. 4. Liver digest:* Fresh rat liver was blended with 0.9 per cent NaCl in the Waring blender and incubated at 37° with a saline extract of Merck pancreatin until the formol titration and material precipitable with trichloroacetic acid became constant. The digest was shaken thoroughly with chloroform to remove protein (2) and lipid. The aqueous phase was ultrafiltered and concentrated in vacuo at 55°. Tyrosine separated during the concentration and was filtered off.

*No. 5. Nuclei:* Nuclei were prepared from perfused rat liver, as described by one of us (3), and then suspended in saline. The suspensions contained from 5 to 7 per cent by volume of packed nuclei.

*Description of table 2. Chromatin:* Liver was blended as described above under "Sediment," table 1, no. 1-no. 4. The suspension was purified by the method of Claude and Potter (4) and usually given one additional washing. In all cases 0.9 per cent NaCl was used as the medium of suspension. Frequently during the blending or the centrifugation the material became granular in appearance.

In preparation 5B the chromatin was lyophilized and stored in air at room temperature before resuspension.

*Description of table 3. No. 1. Ultrafiltrate:* The supernatant fluid from a chromatin preparation was passed through a collodion membrane with a pressure gradient of 15 cm. Hg (5).

*No. 2. Fraction soluble at pH 10.5:* A crude chromatin preparation was brought to pH 10.5 with NaOH and centrifuged one hour at 2,500 r.p.m. The supernatant was adjusted to pH 6.5 with HCl and centrifuged 15 minutes at 2,500 r.p.m.

*No. 3. Fraction soluble in 1 M NaCl:* A chromatin suspension was treated successively with one volume 2 M NaCl and 2 volumes of 1 M NaCl, mixed in the Waring blender and adjusted to pH 8.5. The mixture was centrifuged one hour at 2,500 r.p.m. The supernatant was then decanted into 5 volumes of water and adjusted to pH 7.0. Generally a long fibrous precipitate is obtained, in which case it is blended 3 minutes in the Waring blender. From 25 to 50 per cent of the chromatin could be dissolved. In 3L the preparation was

carried out at room temperature and the material was redissolved and reprecipitated. Preparation 3H was adjusted to pH 10.5 with NaOH and maintained there 30 minutes before readjusting to pH 7.5. It contained visibly larger particles than the others in the table.

TABLE 1  
*Liver fractions other than chromatin*

	PER CENT MITOSIS			DOSE	
	Control	Experimental	Per cent increase	N	P/N
				mgm.	
1. Sediment after 15 minutes at 2,500 r.p.m.....		Toxic			
2. Supernatant of 1.....	1.38	0.90	-35	1.69	0.11
3. Supercel filtrate of 2.....	1.38	0.94	-32	0.78	0.10
4. Pancreatin digest of whole liver.....	0.28	0.20	-28	2.48	0.10
5. Nuclei (citric acid)*					
A.....	0.86	0.71	-17		
B.....	0.86	0.90	4		
C.....	0.86	1.37	59		

\* The dosage in A was 3 injections each containing 0.01 ml. of packed nuclei; in B, 5 injections containing 0.01 ml.; in C, 5 injections containing 0.015 ml.

N = nitrogen; P = phosphorus.

TABLE 2  
*Chromatin from liver*

	PER CENT MITOSIS			DOSE	
	Control	Experimental	Per cent increase	N	P/N
				mgm.	
1. Rat—normal liver					
A. (Pectin control).....	1.06	1.33	25	0.50	0.19
B.....	0.64	1.24	94	0.72	0.10
C. (P <sup>32</sup> control, P <sup>32</sup> in chromatin).....	0.84	1.32	57	1.02	0.12
D.....	0.63	0.55	-13	0.67	0.12
E.....	0.38	0.83	106	0.41	0.14
F.....	0.53	0.65	12	0.17	0.18
G. (P <sup>32</sup> control, P <sup>32</sup> in chromatin).....	0.49	0.62	26	0.72	0.20
2. Rat—regenerating liver.....	0.84	1.59	90	1.04	0.12
3. Beef—normal liver.....	0.47	0.88	70	0.22	0.22
4. Rabbit—normal liver					
A.....	0.38	0.59	55	0.45	0.19
B. (P <sup>32</sup> in chromatin).....	0.23	0.90	290		
5. Rat—various treatments					
A. Prepared at room temperature.....	0.41	0.43	4	0.50	0.20
B. Lyophilized and resuspended.....	0.30	0.33	10	0.58	0.16
C. 1. E stored 3 days.....	0.30	1.39	360	0.41	0.14

No. 4. Fraction insoluble in 1 M of NaCl: The sediment from the first centrifugation in no. 3 above was washed again with 1 M NaCl. In preparation 4E this insoluble material was brought to pH 10.5 and treated as in no. 2 above.

*No. 5. Lipid:* Preparations A and B were made from lyophilized chromatin by Soxhlet extraction with ethyl ether. The lipid extracted was shaken with saline and Solvit A (Emulsol Corp., Minneapolis). Only a small amount could be suspended.

TABLE 3  
*Fractions prepared from whole chromatin*

	PER CENT MITOSIS			DOSE	
	Control	Experimental	Per cent increase	N	P/N
				mgm.	
1. Ultrafiltrate from chromatin suspension.....	0.64	0.66	3	0.005	0.2
2. Soluble in NaOH at pH 10.5.....	0.50	0.75	50	0.45	0.23
3. Soluble in 1 M NaCl					
A. Rat.....	0.94	1.54	64	0.23	0.16
B. A., stored 2 days.....	0.63	0.71	13	0.20	0.16
C. Rat.....	0.56	1.36	143	0.26	0.34
D. C., stored 4 days.....	0.36	0.39	8	0.22	0.34
E. Rat.....	0.30	0.68	129	0.43	0.28
F. Rat.....	0.94	1.12	19		
G. F., incubated 9 hours at 37°.....	0.44	0.57	31		
H. F., adjusted to pH 10.5 for 30 minutes.....	0.94	0.65	-21		
I. Beef.....	0.49	0.59	22	0.03	0.55
J. Rabbit.....	0.38	0.38	0	0.20	0.37
K. Rabbit (containing P <sup>32</sup> ).....	0.23	0.52	126		
L. Rabbit (prepared at room temperature).....	0.86	0.90	5		
4. Insoluble in 1 M NaCl					
A. Rat.....	0.94	0.85	-10	0.17	0.11
B. A., stored 2 days.....	0.63	0.79	25	0.15	0.11
C. Rat.....	0.56	0.65	16	0.17	0.15
D. Beef.....	0.47	0.45	-4	0.10	0.11
E. Rat soluble in NaOH pH 10.5.....	0.30	0.55	84	0.17	0.17
				lipid	
				mgm.	
5. Lipid from rat chromatin					
A. In saline + solvit A.....	0.87	1.24	43	0.4	
B. As A, but from regenerating liver.....	0.87	1.12	29	0.4	
C. In propylene glycol.....	0.91	0.67	-26	8.0	
D. In propylene glycol.....	0.41	0.40	-1	6.3	
E. In propylene glycol.....	0.30	0.52	74	5.0	
F. In propylene glycol (containing P <sup>32</sup> , P <sup>33</sup> control).....	0.49	0.39	-20	4.0	
				N	
				mgm.	
6. Fat free chromatin					
A. P <sup>32</sup> in experimental and control.....	0.49	1.49	200	0.07	0.08
B. Saline extract.....	0.70	0.59	-16	0.04	0.35
7. Protein from chromatin.....	1.02	0.25	-75	0.20	0.08
8. Nucleic acid					
A.....	1.02	0.27	-74	0.08	0.41
B.....	0.53	0.29	-45	0.01	



The other preparations were made by extracting the lyophilized (C, E) alcohol dried (D), or acetone dried (F) chromatin with boiling 50 per cent acetone-50 per cent alcohol (6), filtering, and washing on the filter with ethyl ether. The solvents were removed in vacuo at a temperature of 50°. The lipid was suspended in propylene glycol at a concentration of from 2 to 5 per cent. Some of the lipid dissolved and the remainder was in fine suspension.

*No. 6. Fat free chromatin:* The residue from the alcohol-acetone extraction was dried in vacuo at room temperature. The powder was extracted with an amount of saline corresponding to the usual dilution of chromatin. Preparation A contained those particles small enough to pass a no. 27 needle. Preparation B was the clear supernatant fluid of a centrifuged extract. The solutions were bright lemon yellow.

*No. 7. Protein from chromatin:* Protein from chromatin was obtained by washing a chromatin preparation with 75 per cent saturated  $(\text{NH}_4)_2\text{SO}_4$ . This process removed only part of the phosphorus present.

TABLE 4  
*Miscellaneous substances*

	PER CENT MITOSIS			DOSE
	Control	Experimental	Per cent increase	
1. Commercial intravenous amino acids				
A. Stearn's.....	0.55	0.34	-37	0.2 ml.
B. Stearn's.....	0.76	0.54	-28	0.2 ml.
C. Amigen.....	0.76	0.65	-14	0.2 ml.
2. 1 Cysteine (Pfanstiehl).....	0.55	0.22	-59	3.0 mgm.
3. dl Methionine (SMACO)				
A.....	0.55	0.54	-2	3.5 mgm.
B.....	0.28	0.23	-17	1.5 mgm.
4. Insulin (Lilly).....	0.82	0.58	-29	1.0 unit
5. Adenosine triphosphate (rabbit muscle)....	0.76	0.67	-11	5.6 mgm.
6. Adenylic acid (muscle).....	0.53	0.52	-2	2.2 mgm.
7. Propylene glycol.....	0.36	0.55	51	0.2 ml.
8. Lecithin (Pfanstiehl) in propylene glycol....	0.36	0.41	13	6 mgm. in 0.2 ml.
9. Biotin				
A. 10 $\mu$ gm. i.v. daily 4 days.....	0.97	0.66	-32	
B. 10 $\mu$ gm. i.v. 3 hours before removal.....	0.92	0.76	-17	

*No. 8. Nucleic acid:* The chromatin suspension was saturated with NaCl and filtered. The filtrate was shaken with  $\text{CHCl}_3$  to remove protein (2). Nucleic acid was precipitated with alcohol from the aqueous phase. The precipitate was redissolved and reprecipitated three times. It was then washed with ethyl ether, dried and dissolved in 0.9 per cent NaCl.

Preparation 8B was made from rat liver by digestion as in table 3, no. 4, but in the presence of 0.05M NaF, in order that the desoxyribonucleic acid would not be destroyed (7). The digest was shaken with  $\text{CHCl}_3$  and purified as in 8A.

*Description of table 4. No. 5. Adenosine triphosphate:* Adenosine triphosphate was prepared by Dr. N. O. Kaplan according to the method of Needham (8) from a rabbit anesthetized with  $\text{MgSO}_4$  (9).

*No. 6. Adenylic acid:* Adenosine triphosphate was hydrolyzed with an enzyme in the albumin fraction of potato juice (10). The split products were purified according to the method of Kerr (11).

**RESULTS.** To determine whether the mitotic rate in the regenerating liver was influenced by other factors than the test substances, the following experiments were performed:

1. *Anesthesia.* Twenty-four hours after partial hepatectomy, animals were treated with ether to produce deep anesthesia for 20, 15 and 5 minutes respectively. Controls received no ether. The mitotic rates observed 3 hours later were 0.42, 0.50 and 0.50 per cent respectively for each of the 3 rats, while the rats in the 3 controls were 0.37, 0.44 and 0.38 per cent. None of these differences is significant.

2. *Distribution of mitotic figures in centrifugation.* Nuclei were isolated in the usual way by centrifugation after treatment with 5 per cent citric acid. Samples of the packed nuclei were taken from 4 successive depths in the centrifuge tube. Mitotic counts from each of these layers beginning at the top were 32, 41, 38 and 41 respectively. There was, therefore, an approximately uniform distribution of mitosis.

3. *Radioactive phosphorus.* Since  $P^{32}$  was contained in some of the preparations, and also used in some of the controls, the effect of radioactivity on mitosis was determined. With the exception of one experiment to be described later, the dose of radioactive phosphorus administered was from 1 to 8  $\mu$ c. per animal. Within this range there was no significant effect on mitosis. For example, 7.6  $\mu$ c. was given subcutaneously after partial hepatectomy and livers removed 24 hours later showed 0.82 per cent mitosis, while those of controls to which saline had been administered showed 0.85 per cent. In another experiment, as much as 30  $\mu$ c. per rat was given intravenously 24 hours after partial hepatectomy and the livers were removed 3 hours later. The mitotic rate was 0.49 per cent, while in the control, which in this case received lipid in propylene glycol, it was 0.39 per cent.

Table 1 shows the effect on mitosis of various fractions from the liver other than chromatin. All retarded mitosis with the exception of injections of whole nuclei. The latter were prepared by the use of 5 per cent citric acid in the same manner as those obtained for mitotic counts.

In table 2 it will be seen that, with two exceptions, chromatin increased the rate of mitosis in the liver. Changes of 10 per cent or less are within the limits of error in making the mitotic count and probably are not significant. Material prepared at room temperature had little or no effect. Although chromatin which had been lyophilized had no activity this is not necessarily the result of lyophilization since the preparation was exposed to air at room temperature after lyophilization. The one negative result 1D is the average of individual counts from only 3 animals in the control and experimental groups. The variation in the control groups was exceptionally high, 0.5 to 1.5 per cent. Since 9 out of 11 preparations increased the mitotic rate by 25 per cent or more when as little as 2 mgm. protein was given a rat weighing 50 grams, the results provide a definite indication of the presence of a mitosis-stimulating substance in the chromatin. This is not species-specific since chromatin from beef or rabbit livers increases the mitotic rate in the rat.

The stimulating substance is not present in the ultrafiltrate from chromatin but does appear in the fraction soluble in 1 M NaCl (table 3). As with chromatin, preparation at room temperature produced only inactive material. Of 7 preparations of the 1 M NaCl extract from different sources, only one (rabbit) failed to show a positive effect on mitosis. When a second preparation of rabbit chromatin was made, it was found to have strong mitotic activity. The fraction insoluble in 1 M NaCl does not stimulate mitosis. An experiment was carried out to determine whether increasing doses of the soluble and insoluble fraction produced increasing effects on mitosis. Six rats were used, each of 3 receiving 0.1, 0.2 and 0.4 ml. respectively of the soluble fraction and each of 3 others equivalent amounts of the insoluble fraction. The results were as follows:

<i>ml. Injected</i>	<i>Soluble</i>	<i>Per cent Mitosis</i>	<i>Insoluble</i>
0.1	1.4		1.1
0.2	1.5		0.9
0.4	1.6		0.6

Apparently increasing amounts of the soluble fraction produced progressive increases in mitosis while increasing amounts of the insoluble portion decreased mitosis.

Chromatin lipid emulsified in saline had a slight positive effect on mitosis. With one exception the results were negative when the lipid was suspended in propylene glycol alone. The controls in this case received intravenous saline. Since propylene glycol had some stimulating effect, the exceptional value obtained with the lipid in propylene glycol cannot be considered evidence of the stimulation of mitosis by lipid. Similarly, in the case of saline emulsions, the positive results may possibly have been caused by the emulsifying agent for which no control experiments were run. In any case, since equal numbers of positive and negative effects were obtained, the lipid from chromatin cannot be considered the mitosis-stimulating agent.

Nucleic acid and protein obtained from chromatin both reduced the mitotic rate. Liver digest, commercial intravenous amino acid preparations, methionine, cysteine, adenosine triphosphate and biotin all had either no effect or an inhibitory one. A saline extract of fat free chromatin had a slight negative effect, although the fat free chromatin itself gave a marked positive effect (200 per cent increase after injection of less than 0.5 mgm. of protein).

The increase in the number of nuclei in metaphase and anaphase is not in itself sufficient evidence for an increase in the rate of mitosis, for such a result may be obtained if mitosis is arrested at either of these stages. Counts were therefore made of the relative frequency of nuclei in all stages of the mitotic cycle, i.e., resting stage, prophase, metaphase, anaphase and telophase. The proportion of nuclei in anaphase as compared to metaphase, and of both these stages to pro-phases in chromatin treated animals was found to be the same as in the controls. It was concluded therefore that the observed increase in the frequency of meta-

phases and anaphases was due to a true increase in the rate of mitosis, and that the phase of the nuclear cycle in which the stimulation occurred was some portion of the resting stage.

Since 9 of 11 crude chromatin preparations, 6 of 7 NaCl extracts of chromatin and fat free chromatin produced a marked increase in the rate of division of cells in the liver, while other fractions derived from liver inhibited mitosis, it seems clear that chromatin contains a mitosis-stimulating component.

**DISCUSSION.** The preparations used in these experiments are similar to but not necessarily identical with some previously described. From leukemic cells Claude and Potter (4) prepared chromatin which was white, in contrast to our chromatin from liver which is brown. They found only 2.3 per cent of lipid in the chromatin, which they considered might be entirely due to contamination by cytoplasmic constituents. When extracted with hot alcohol-acetone mixture, liver chromatin yields 20 per cent of lipid by weight, while Soxhlet extraction yields only 10 per cent. The large quantity of lipid and the different yields obtained by the two methods of extraction indicate that a good portion of the lipid is bound as an integral part of the chromatin. The portion of the chromatin soluble in 1 M NaCl is similar to material described by Bensley (12) and by Mirsky and Pollister (13). Although described as nucleoprotein by the latter investigators, our results show it to be more complex since it contains lipid as a constituent. Stedman and Stedman (14) described a substance "chromosomin" obtained from nuclei, but, since neither an adequate description of its properties nor the method for its preparation were given, no comparison could be made with the substances reported here. None of the investigators mentioned studied the physiological properties of the substances they described.

Since it has been shown (15) that insulin increases the amount of adenosine triphosphate in the liver, the failure of insulin to increase the mitotic rate may be taken as evidence that mitotic stimulation by chromatin and some of its fractions cannot be attributed to adenosine triphosphate. The latter was considered by Loofbourow to be a possible factor in stimulating the growth of yeast cells (16).

The presence in chromatin of the two substances, one of which stimulates mitosis while the other inhibits it, is of particular interest in an understanding of the physiology of the nucleus. The stimulation observed must be due to chromatin or a portion thereof, but cannot be ascribed to ordinary nutrients that chromatin may contain, since nucleic acids and amino acids produced no such effect. Neither can the mere particulate nature of the chromatin be held responsible since suspensions of the particulate material from the liver or even chromatin prepared at room temperature had an opposite effect or none at all.

#### SUMMARY

1. Mitosis in regenerating liver is increased by intravenous injections of small amounts of chromatin, fat free chromatin, and that portion of chromatin which is soluble in 1 M NaCl.

2. Mitosis is inhibited by other fractions from liver and by that portion of chromatin which is insoluble in 1 M NaCl.

3. Amino acids, nucleic acid, lipid, biotin, adenosine triphosphate and insulin have either no effect or an inhibitory one on mitosis.

4. Chromatin contains some factor which stimulates mitosis.

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## TRANSFER OF $P^{32}$ FROM INTRAVENOUS CHROMATIN TO HEPATIC NUCLEI<sup>1</sup>

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In some experiments (1) in which the rate of mitosis of cells in regenerating liver tissue was measured, an apparently significant increase in this rate was produced by the injection of chromatin strands (2) and liponucleoprotein prepared from the strands. Bensley (3), who made a similar preparation, considered this material to be of cytoplasmic origin and described its lipid but not its nucleic acid content. Mirsky and Pollister (4) considered this material to be of nuclear origin and described its nucleic acid but not its lipid content.

Since the chromatin and some of its derivatives were injected as particulate matter easily visible under the microscope, it was difficult to understand how this material could influence the rate of mitosis. Experiments were designed to determine how much, if any, actually entered the liver cell. Chromatin was prepared from perfused livers of animals which had received  $P^{32}$  as  $Na_2HPO_4$  24 hours earlier. Twenty-four hours after partial hepatectomy, suspensions of this chromatin (0.2 ml.) were injected intravenously in rats weighing 50 grams. Three hours after the injection samples of perfused liver were removed for  $P^{32}$  assay and for isolation of nuclei. The  $P^{32}$  in perfused liver tissue and in liver nuclei was measured with a Geiger-Muller counter. In two preliminary experiments, nuclei showed a much greater uptake of  $P^{32}$  from chromatin than from  $PO_4$ , but the results were discarded since the readings were too close to background to be reliable. These experiments were then repeated, once with rabbit chromatin and once with rat chromatin. In the former, nuclei readings were 50 per cent above background or 10 times the mean deviation of readings in any one group. In the latter the radioactivity in the nuclei was 40 times the background. As tables 1 and 2 show, there is good agreement in the results of both experiments. Two values are given for inorganic phosphate, the first is an average of 19 experiments, the second is the mean for 10 rats used as controls for the experiment with radioactive rat chromatin and its derivatives.

The retention of  $P^{32}$  by liver tissue three hours after injection is more than 5 times as great following administration of labelled chromatin or lipid from chromatin than the retention of phosphate ion. With both of these substances 50 per cent of the entire dose is held by the liver, as compared with 9 per cent with  $PO_4$ . The suspension of fat free chromatin showed a lower percentage of retention than chromatin but one which was still significantly higher than the retention of inorganic phosphate. Since, in this case, a considerable amount of

<sup>1</sup> The work described in this paper was done under a contract recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the University of California.

the  $P^{32}$  was present in coarse particles which settled in the lungs (scattered capillary emboli were produced) the retention by the liver of material which actually reached it was considerably greater than the 7 per cent indicated in table 1.

To determine whether the radioactive material held by the liver tissue was actually being utilized by the liver cells, the nuclei were carefully isolated and the uptake per gram of nuclei was determined as described by Marshak (5). These results are shown in table 2. There was more than three times as much  $P^{32}$  per gram of nuclei in animals which received chromatin or fractions thereof than in those which received inorganic phosphate. Obviously, material from chromatin does enter the nuclei. From the estimate that nuclei constitute 6 per cent of the liver tissue (5) the  $P^{32}$  in the nuclei may be calculated as a percentage

TABLE 1

*$P^{32}$  in regenerating livers three hours after intravenous injection of labelled substances*

MATERIAL INJECTED	NUMBER OF RATS	$P^{32}$ /GRAM LIVER AS % DOSE	STANDARD ERROR OF MEAN
1. Inorganic phosphate.....	84	4.52	0.083
2. Inorganic phosphate.....	10	4.36	0.084
3. Rat chromatin.....	19	26.30	0.670
4. Lipid (rat chromatin).....	15	32.20	0.390
5. Fat free chromatin (rat).....	5	7.10	0.480
6. Adenosine triphosphate.....	7	4.43	0.320
7. Rabbit chromatin.....	8	23.10	0.200
8. Rabbit chromatin soluble in 1 M NaCl.....	8	16.20	0.470

TABLE 2

*$P^{32}$  uptake by nuclei*

SUBSTANCE INJECTED	PER CENT DOSE /GRAM NUCLEI	PER CENT LIVER $P^{32}$ IN NUCLEI
Inorganic phosphate.....	1.54	2.1
Rat chromatin.....	5.08	1.2
Rabbit chromatin.....	5.06	1.3
Rabbit chromatin soluble in 1 M NaCl.....	4.23	1.6
Fat free chromatin (rat).....	5.45	4.6
Lipid (rat chromatin).....	5.15	1.0

of the total radioactivity of the liver. These results are given in column 3. When considered thus it appears that  $P^{32}$  enters the nuclei least readily from lipid, less from crude chromatin than from  $PO_4$ , and most efficiently from fat free chromatin. Thus the greater nuclear  $P^{32}$  uptake from chromatin cannot be attributed merely to the larger amount retained by the liver but to a more rapid transfer to the nucleus.

DISCUSSION. From these experiments with  $P^{32}$  it is clear that the particulate nature of the chromatin injected is responsible for its greater retention by the liver. The chromatin, however, is not held there merely as inert material, for the results show that in some way either the whole chromatin or parts of it become incorporated into the liver nuclei since the terminal  $PO_4$  groups of

the chromatin cannot alone account for the nuclear uptake of  $P^{32}$ . Circumstances made it impossible to carry out a control experiment, as had been done earlier for inorganic phosphate (5), to determine whether or not the nuclear radioactivity might be due to contamination. However, the repeated washings in the isolation of the nuclei, the consistency of the results with chromatin, and the characteristically different amounts of  $P^{32}$  reaching the nuclei from the different fractions of chromatin all make it very unlikely that the nuclear  $P^{32}$  is due to contamination.

The absence of any difference in the uptake of  $P^{32}$  between adenosine triphosphate and inorganic phosphate is in accord with the results of Furchgott and Shorr (6), who found that extracellular inorganic phosphate exchanges directly with the intracellular phosphate and that the terminal phosphate of adenosine triphosphate has the same specific activity as the intracellular inorganic phosphates.

In previous experiments by one of us (5) it was shown that the chromatin of the living nucleus is in a state of dynamic equilibrium in which portions of its nucleoprotein are constantly being removed and replaced. Since, in the experiments being reported here, the  $P^{32}$  from intravenous chromatin was incorporated rapidly into the nucleus, the present results suggest that this chromatin participates in the same or similar reactions. It seems probable that the alteration in type of pneumococcus cultures described by Avery et al. (7) may have been produced by an analogous process, although Sonneborn (8) has attributed such changes to modification of the cytoplasm. The possibility that extracellular nucleic acid or nucleoprotein may become built into the nucleus by this mechanism of physiological exchange carries with it far reaching implications in cellular physiology and in genetics. For example replacement of part of the nucleoprotein at any one locus in the chromosome by a similar but slightly different portion from the extracellular chromatin may lead to a change in function at the locus comparable to a gene mutation. The result would be similar to that obtained for pneumococci treated with nucleic acid.

#### SUMMARY

1. A remarkably large fraction of particulate materials derived from chromatin labelled with  $P^{32}$  is retained by the liver 3 hours after injection into the femoral vein.

2. It is demonstrated that either whole chromatin or portions thereof become incorporated more rapidly into liver nuclei than does inorganic phosphate.

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# HYPOPROTHROMBINEMIA INDUCED IN SUCKLING RATS BY FEEDING 3,3'-METHYLENEBIS(4-HYDROXYCOUMARIN) AND ACETYLSALICYLIC ACID TO THEIR MOTHERS<sup>1</sup>

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The status of our knowledge on the transmission of drugs and poisons through the milk of lactating animals has been summarized in Rogers (1, p. 590-591) in the work of Hatcher and associates (2) and by Joachimovits (3). While there appears to be little or no general tendency for drugs and poisons to be excreted in the milk, it is recognized that small quantities of aspirin, calomel, arsenic, iodides and bromides appear in the milk subsequent to the administration of therapeutic doses of these drugs. It has also been reported that barbiturates may appear in human milk (4, 5).

When cattle eat the white snake root (*Eupatorium urticaefolium*) or the rayless golden rod (*Aplopappus heterophyllus*) they usually succumb to the disease known in veterinary practice as "trembles" (1). It is claimed that if the milk from cows that have eaten these toxic plants is consumed by man, symptoms comparable to cattle "trembles" develop (1). That the agent responsible for the hemorrhagic sweet clover disease of cattle might also enter into the milk is suggested by an observation of Roderick (6, p. 33-35; 7) who noted that calves from cows who were consuming improperly cured sweet clover hay at parturition succumbed with the characteristic hemorrhages of the disease. Since the calves had appeared physically normal at birth and had died before they were old enough to eat the damaged hay, Roderick suspected that the hemorrhagic agent might have been transmitted through the milk. This point of view was expressed to Professor Link by Doctor Roderick in a private conversation here in July, 1943.

Shortly after the causative agent of the hemorrhagic sweet clover disease was identified as 3,3'-methylenebis(4-hydroxycoumarin)<sup>2</sup> (8) it was observed by Field, Overman and Baumann (9) that pregnant and lactating rats tolerate higher levels of the anticoagulant than normal females. In an extension of this study it was noted that the continuous feeding of the anticoagulant to female rats with suckling pups caused hemorrhages to appear in the young. The present

<sup>1</sup> Published with the approval of the director of the Wisconsin Agricultural Experiment Station. This work was supported through special grants from the Graduate Research Committee of the University and the Wisconsin Alumni Research Foundation.

This paper represents a phase of the studies on the anticoagulant 3,3'-methylenebis(4-hydroxycoumarin) carried out in the laboratory of Prof. Karl Paul Link (see the Harvey Lectures Series XXXIX, 162-216 (1943-44)). I wish to acknowledge my indebtedness to Professor Link and to Dr. C. A. Baumann for the counsel and guidance they gave me in conjunction with this work.

<sup>2</sup> Available to the clinician under the trademark *Dicumarol*.

paper deals with the quantitative measurement of the hypoprothrombinemia that develops in female rats and their suckling pups when the anticoagulant, 3,3'-methylenebis(4-hydroxycoumarin) is given to the mother. Comparable observations were made on the hypoprothrombinemia detectable when large doses of acetylsalicylic acid are fed to nursing rats (10).

**METHODS.** Several days prior to parturition pregnant female rats were placed on our standard semi-synthetic diet (11) which is deficient in vitamin K. In no case was any experiment begun until the diet had been fed for at least 5 days. This interval appears to be sufficient to remove the protective effects provided by vitamin K and other antihemorrhagic substances in the previous diet (11). Shortly after birth the litters were reduced to 6 to 8 young. The experiments were begun when the pups were about 3 or 4 days old. The food of the mother was not accessible to the pups, hence the only nourishment obtained by the young was through the milk of the mother. Blood samples were obtained at the same time from the mother and 3 of her litter by cardiac puncture under light ether anesthesia. A sample of 0.9 ml. blood was withdrawn from the mother into 0.1 M sodium oxalate solution, while 0.45 ml. blood from the pups was withdrawn into 0.05 ml. of the oxalate solution.<sup>3</sup> The prothrombin time of the plasma diluted to 12.5 per cent was determined by the procedure developed in this laboratory (12). In control studies not included here, the prothrombin time of whole plasma (100 per cent) and the 50, 25 and 6.25 per cent dilutions was also measured. But the data will be restricted to the prothrombin time of 12.5 per cent plasma (1 part plasma, 7 parts saline solution) inasmuch as the change in prothrombin activity reflected by plasma of this dilution is readily detectable and reproducible (12).

The anticoagulant was mixed directly with the food of the mothers while the acetylsalicylic acid was administered by stomach tube as a suspension in 1 per cent gum tragacanth solution.

**EXPERIMENTAL.** *Hemorrhages in suckling pups from mothers fed 3,3'-methylenebis(4-hydroxycoumarin).* After feeding 5 mgm. of 3,3'-methylenebis(4-hydroxycoumarin) daily for about 4 days to lactating rats, visible hemorrhages appeared in some of the suckling young. The pups would at first show petechiae at the extremities, and the growth curve would plateau. After feeding the anticoagulant for 5 to 6 days, spontaneous hemorrhages could be seen in other regions of the pups while some died from the hemorrhage. Pools of blood accumulated in the abdominal cavity, and spontaneous hemorrhages appeared in the testes. The most frequent site of hemorrhagic lesions was the spinal canal and region of

<sup>3</sup> It should be indicated that the difficulty of maintaining a significant number of pups of the same litter through the experimental period of 4 to 8 days could be overcome by sampling only 3 pups at one time. It was anticipated that a technique which involved the cardiac puncture of young pups (8-10 grams) and the withdrawal of 50-60 per cent of the circulating blood, usually hypoprothrombinemic, would result in a high incidence of fatality. However, the over-all fatality (10 per cent) was less than expected. It was found that the prothrombin time of a pup two days after sampling was unchanged from the first sample. Thus, when all pups of a litter had been bled once, those surviving the first sampling were available for the second sample.

the medulla (11). Visible hemorrhages developed in approximately 50 per cent of the young. In general, the gross lesions were very similar to those reported for cattle and the rabbit after the ingestion of spoiled sweet clover hay (6, 7). The mothers would always succumb to a regime of 5 mgm. of the anticoagulant fed daily for 6 to 9 days with a widespread and typical diathesis.

*Hypoprothrombinemia in suckling pups caused by feeding 3,3'-methylenebis(4-hydroxycoumarin) to their mothers.* The prothrombin time of 12.5 per cent plasma from large numbers of normal rats has been found to be about 40 seconds (9, 11). The clotting time of 12.5 per cent plasma from lactating mothers was likewise 40 seconds and that of their suckling young was 37 seconds (table 1). When 5 mgm. of the anticoagulant were fed to the mother, the prothrombin times of the plasma from both the mother and young was prolonged above the normal value. After 1 day of feeding, the average 12.5 per cent prothrombin times of the mothers rose to 77 seconds, while that of the pups increased to 84 seconds (table 1). After 2 consecutive daily feedings of 5 mgm. of the anticoagulant, the prothrombin time of the mothers was 125 seconds, that of the pups, 121 seconds.

TABLE 1

*Effect of feeding 5 mgm. of 3,3'-methylenebis(4-hydroxycoumarin) daily to lactating rats on the prothrombin time of the mothers and their suckling pups*

	PROTHROMBIN TIME IN SECONDS OF 12.5 PER CENT PLASMA										NO. OF RATS
	Normal		1 day		2 days		3 days		4 days		
	Average	Range	Average	Range	Average	Range	Average	Range	Average	Range	
Mothers.....	40	36-45	77	71-83	125	115-134	>300	>300	>300	>300	7
Pups.....	37	30-43	84	57-109	121	77->300	>300	>300	>300	>300	42

After the mothers had consumed the anticoagulant for three consecutive days, a severe state of hypoprothrombinemia existed. Prothrombin times of 300 seconds or more were noted for the plasma from both the mothers and the pups on whole plasma as well as the 12.5 per cent plasma. Such a drastic prolongation of the prothrombin time of the whole plasma indicates that the percentage reduction in prothrombin activity approximates 97 per cent of the normal (12, 13).

*Effect of acetylsalicylic acid.* It was demonstrated in this laboratory that the widely used salicylates induced hypoprothrombinemia in rats (10). Clinical workers subsequently were able to show that the salicylates evoke the same response in man (14-17). Perhaps the most active anticoagulant of the salicylate class is acetylsalicylic acid (aspirin). Therefore, lactating rats were given a 100 mgm. dose of aspirin daily in suspension by stomach tube, and at suitable intervals blood samples were withdrawn from both mother and young. A definite hypoprothrombinemia developed in both the mothers and in the suckling pups (table 2). The hypoprothrombinemia was similar to that caused by 3,3'-methylenebis(4-hydroxycoumarin) although of a reduced intensity.

*Protective effect of vitamin K.* The protective capacity of various forms of

vitamin K against the action of the anticoagulant in the rat has already been reported in detail (10, 11). Consequently, the actual quantitative figures of the experiments under consideration need not be given. When 10 mgm. of 2-methyl-1,4-naphthoquinone<sup>4</sup> were fed to the mothers daily simultaneously with 5 mgm. of the anticoagulant a border line and variable protective action was noted, with 25 mgm. of the naphthoquinone the protective action became more regular, while with 50 mgm. it was not only quite marked but fairly consistent. Thus the average prothrombin time of 12.5 per cent plasma of mothers on the 5th day was 133 as opposed to over 300 seconds without the naphthoquinone (see table 1) while that of the pups was 54 seconds. In other words, the protective effect was more apparent in the pup than in the mother. It should be indicated, however, that vitamin K cannot protect the rat indefinitely against large doses of the anticoagulant (11).<sup>5</sup>

DISCUSSION. The experiments have demonstrated that when the anticoagulant 3,3'-methylenebis(4-hydroxycoumarin) and acetylsalicylic acid are fed to lactating rats, their suckling pups become hypoprothrombinemic and sub-

TABLE 2

*Effect of feeding 100 mgm. of acetylsalicylic acid daily to lactating rats on the prothrombin time of the mothers and their suckling pups*

	PROTHROMBIN TIME IN SECONDS OF 12.5 PER CENT PLASMA										NO. OF RATS
	Normal		1 day		2 days		3 days		5 days		
	Average	Range	Average	Range	Average	Range	Average	Range	Average	Range	
Mothers.....	40	37-42	45	41-50	49	38-61	77	49-104	55	51-58	7
Pups.....	37	30-43	49	38-59	47	37-61	53	40-81	47	38-55	34

ject to hemorrhage. Although it had previously been noted that salicylates may be transported through the milk (1, 2) the possibility of an adverse effect on the infant has not been emphasized.

It is not known at present whether the 3,3'-methylenebis(4-hydroxycoumarin) fed to the suckling female rat passes directly into the milk or whether an active metabolite from it is the cause of the hypoprothrombinemia and hemorrhage in the suckling young. Although it would appear that the mammary gland is permeable to vitamin K, it cannot now be stated that its protective action is due to the transmission of the intact molecule or to that of an active metabolite of the vitamin.

<sup>4</sup> The vitamin K (Menadione-Abbott) was kindly supplied by Dr. Carl Nielsen, Abbott Laboratories, North Chicago, Illinois.

<sup>5</sup> We have recently shown (18) that the methylxanthines, caffeine, theobromine and theophylline, reduce the extent of the hypoprothrombinemia induced by 3,3'-methylenebis(4-hydroxycoumarin) in the dog and rat. Lactating rats receiving 5 mgm. of the anticoagulant daily, were also given 100 mgm. theobromine daily. The prothrombin times of both mothers and suckling rats were significantly shorter than those of the controls given the anticoagulant alone.

Davis and Porter (19) have recently reported favorable clinical results in the treatment of puerperal thrombosis with 3,3'-methylenebis(4-hydroxycoumarin). It would appear that when the anticoagulant is used in this disorder the suckling infant should either be withdrawn from the mother when the drug is given, or the prophylactic administration of vitamin K to the infant be considered.

#### SUMMARY

Hypoprothrombinemia and hemorrhage can be induced in suckling rat pups and rats given the anticoagulant, 3,3'-methylenebis(4-hydroxycoumarin). By administering vitamin K to the mother the extent of the hypoprothrombinemia induced in the pups was markedly reduced. Hypoprothrombinemia can also be induced in suckling rat pups by giving large quantities of acetylsalicylic acid to the mother. The possible bearing of these findings on the clinical use of 3,3'-methylenebis(4-hydroxycoumarin) is indicated.

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# THE RÔLE OF THEBESIAN DRAINAGE IN THE DYNAMICS OF CORONARY FLOW<sup>1</sup>

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In previous work from this laboratory (1, 2) it was shown that the partition of coronary drainage between the coronary sinus and other channels is variable. In fact, under certain conditions these accessory channels may actually serve as portals for the entrance of blood into the coronary system (1). At that time it was considered that these channels were mainly Thebesian channels, although we recognized that they included accessory coronary veins draining directly into the right auricle. Recently Gregg, Shipley and Bidder (3) investigated the flow in these accessory veins, which are chiefly anterior cardiac veins, and found that the flow through them was large. These investigators concluded that aside from the coronary sinus, the preponderance of drainage channels in the right heart over the left, which we demonstrated, was largely accounted for by these anterior cardiac veins draining into the right auricle. At any rate, their work demanded that a more detailed analysis of drainage of coronary flow should be undertaken. Therefore, we have attempted to measure the five components, viz: *a*, coronary sinus; *b*, other drainage into right auricle (including that of the anterior cardiac veins); *c*, drainage into the right ventricle; *d*, drainage into the left auricle, and *e*, drainage into the left ventricle. This turned out to be a difficult technical problem. It was finally solved, in a manner sufficiently satisfactory for our purpose, using hearts taken from dogs immediately after they were sacrificed. In these hearts, aside from the customary drainage of the coronary sinus by a Morawitz cannula, the auricles were separated from the ventricles at the level of the A-V orifices by specially constructed umbrella-like expanding partitions. These six-prong expanding devices, attached at both ends to a central shaft (fig. 1), were covered by thin readily expandible rubber tubing and inserted into the A-V orifices from above via the superior vena cava and a pulmonary vein. When appropriately placed in the A-V openings they were expanded by a controlling nut on the distal end of the central shaft until the orifices were sealed. To avoid injury to the muscle minimal expansion was used. The coronary arteries were then perfused and the five components of the drainage measured and the percentage distribution of the drainage was calculated.

**PROCEDURE.** Dogs were anaesthetized with nembutal intravenously (25 mgm./kilo.) and heparinized (3 cc. of Liquaemin intravenously<sup>2</sup>). The animals were then sacrificed by using a lethal dose of nembutal or sodium cyanide intra-

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<sup>2</sup> We are grateful to Roche-Organon, Inc., for supplying the Liquaemin.

venously. The chest was opened and the heart and lungs removed *en bloc*. The lungs, esophagus, and trachea were separated by blunt dissection. The aorta, the azygos vein, and a pulmonary vein were cannulated, the former for perfusion of the coronary arteries, the latter for drainage of the right and left auricles respectively. The special umbrella-partitions were inserted via the superior vena cava and a pulmonary vein so that the expansile part lay in the A-V orifice. The umbrellas were then expanded until the orifices were completely occluded. The veins through which they were inserted were tied to the shafts of the two devices. Drainage of the right ventricle was accomplished by cannulating the pulmonary artery (dissection was avoided by using a Morawitz type of cannula). The left ventricle was drained via a cannula inserted through a stab wound at the apex. Finally, a Morawitz cannula was introduced through the inferior vena cava to drain the coronary sinus. The remaining

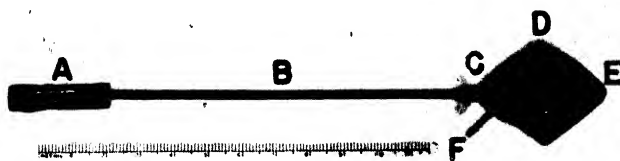


Fig. 1. Photograph of the adjustable device for occluding the A-V ring orifice. The expansible, occluding device consists of six ribs which are hinged at *C*, *D*, and *E*. The double "umbrella" they form is covered by a thin rubber membrane. *B* is a hollow tube fastened to the "umbrella" at *C*. The other end of this tube rests against, but is not fastened to, the threaded nut, *A*. This nut screws on to a central shaft which runs through *B* and is fastened to the other end of the "umbrella" at *E*. Thus, by turning *A* the central shaft moves through *B* causing *E* to move toward or away from *C*, depending on the direction *A* is rotated, and the diameter of the "umbrella" at *D* is varied. The six ribs are maintained equally spaced about the shaft by a thick rubber band, *F*, which is tightly threaded through all six ribs close to *D*.

pulmonary veins were tied off. The five drainage cannulae emptied into graduates by means of rubber tubing. The heart was placed on a wire mesh over a basin to collect leakage, which was later measured.

The coronary system was perfused, via the cannula in the ascending aorta, at a pressure of 150 mm. Hg with 25 per cent dog serum in physiological NaCl, or 100 per cent dog serum at room temperature and the drainage measured. The A-V partitions were checked for competence by obstructing the outflow to one of the chambers in each side of the heart and noting whether or not this affected the flow from the other chamber. Absence of leakage past the aortic valves was demonstrated by the absence of large drainage from the left ventricle. The amount of cardiac edema which developed was measured by comparing the heart weights before and after perfusion.

**RESULTS.** The data on 18 trials in 8 preparations are summarized in table 1

in percentages together with the mean values and standard deviations. The leakage varied from 1 to 7.8 per cent, averaging 3.6 per cent. The drainage percentages were calculated on the basis of the total drainage in each perfusion, exclusive of the leakage, as 100 per cent.

The drainage distribution between the right and left heart in these experiments is close to that observed in our previous experiments (2) on surviving hearts

TABLE 1  
*Partition of coronary flow drainage*

NO.	CORONARY SINUS	RIGHT AURICLE	RIGHT VENTRICLE	LEFT AURICLE	LEFT VENTRICLE	RIGHT HEART	LEFT HEART
	%	%	%	%	%	%	%
1	53.7	17.0	25.8	0	3.5	96.5	3.5
2	26.7	33.3	25.8	0	14.2	85.8	14.2
	23.9	32.5	32.0	0	11.5	88.4	11.5
3	18.2	27.7	44.4	1.2	8.6	90.3	9.8
	27.9	26.4	35.7	0	10.0	90.0	10.0
4	37.8	24.3	27.0	6.8	4.1	89.1	10.9
	28.8	26.7	32.1	2.8	9.6	87.6	12.4
5	35.4	29.1	30.7	2.4	2.4	95.2	4.8
	39.0	26.9	28.4	2.8	2.8	94.3	5.6
	45.3	26.0	22.2	0.8	5.5	92.5	6.6
6	45.3	12.7	32.4	0.3	9.2	90.4	9.5
	47.5	14.7	26.8	1.3	9.7	89.0	11.0
7	52.6	17.3	25.6	1.5	3.0	95.5	4.5
	57.2	14.0	21.8	1.5	5.5	93.0	7.0
8	21.5	31.5	39.0	0	8.2	92	8.2
	20.2	31.6	38.9	0	9.3	90.7	9.3
	39.6	23.4	30.6	0.5	6.3	93.6	6.8
	34.5	25.1	33.9	3.4	3.1	93.5	6.5
Mean.....	36.4	24.5	30.8	1.4	7.0	91.6	8.4
Standard deviation.....	11.8	6.5	5.4	1.7	3.3		

with perfused coronary vessels. In the left heart our results show that most of the small drainage is into the ventricle, indicating Thebesian drainage. In the right heart, the partition between the coronary sinus, other channels draining into the right auricle, and channels into the right ventricle are of the same order of magnitude, namely, 36.4 per cent, 24.5 per cent and 30.8 per cent respectively of the total coronary drainage. This would definitely show that the Thebesians play an important rôle since no veins have been shown to empty directly into



the right ventricle. Even in the case of the right auricle, it may be questioned if all of the extra-sinus drainage represents drainage via the anterior cardiac veins. Some may very well be by way of Thebesian channels. Our results, therefore, establish the fact suggested by our previous results, namely, that Thebesian drainage into the right ventricle is an important element in coronary drainage. Furthermore, it supports our idea, previously expressed, that these Thebesians in the right ventricle may serve as immediate portals of entry of blood when the coronary arteries feeding the right ventricle are suddenly or gradually occluded.

These results are not to be considered quantitatively accurate, since repeated observations on the same preparation show fairly wide variations. The averages of the component drainages differ sufficiently so that qualitative conclusions can, however, be drawn. The variability in data is due to errors inherent in the method of measurement, viz., 1, the possibility of a shift in the A-V partitions permitting slight leaks between the chambers, even though when checked leaks are absent, and 2, the development of cardiac edema and compression of the myocardium by the partitions might alter the distribution of drainage of coronary blood. Although these and other errors may prevent these results from being quantitatively accurate, they do not nullify the conclusion that a significant right ventricular Thebesian drainage occurs.

#### SUMMARY

1. The coronary arteries were perfused in dogs' hearts obtained immediately after the animals were sacrificed, and the drainage into the coronary sinus, right auricle, right ventricle, left auricle, and left ventricle were measured. Special umbrella-like partitions were used to seal the A-V openings.

2. The averages and standard deviation of the percentage flows obtained were: coronary sinus, 36.4 per cent  $\pm$  11.8 per cent; the right auricle, 24.5 per cent  $\pm$  6.5 per cent; the right ventricle, 30.8 per cent  $\pm$  5.4 per cent; the left auricle, 1.4 per cent  $\pm$  1.7 per cent; the left ventricle, 7.0 per cent  $\pm$  3.3 per cent.

3. Details of technique and errors of method were discussed.

4. The flow into the right ventricle via the Thebesian channels was shown to be of significant magnitude.

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# BLOOD VOLUME IN EXPERIMENTAL HEMORRHAGIC SHOCK<sup>1,2</sup>

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It has become increasingly apparent that reduction in blood volume is an invariable concomitant of shock developing from hemorrhage, trauma, and burns. The present series of experiments was undertaken in an attempt to evaluate the magnitude of the blood volume reduction necessary to cause fatal shock in the absence of such modifying factors as tissue injury, pain, or anesthesia.

The problem was approached through studies of unanesthetized dogs in which the blood volume was reduced by bleeding to a critical level such that it was either just adequate to sustain life and permit spontaneous recovery, or was inadequate and led to progressive fatal shock. The emphasis in this paper, then, is not on the volume of blood lost but on the adequacy of the volume remaining after physiological compensation.

**METHODS.** Twenty-three healthy adult dogs, unselected as to breed or sex, were used. The animals were given water but no food for 18 to 24 hours prior to experimentation. The average body weight was 11.1 kgm. (range 7.8 to 15.3 kgm.).

No general anesthetic was employed. The dogs were placed in the supine position on a standard padded animal board. Both femoral arteries, one for bleeding and recording blood pressure and the other for withdrawing blood samples, were exposed under local procaine hydrochloride supported by Neothol.<sup>3</sup> The bleeding consisted of a single massive hemorrhage, averaging 50 per cent of the blood volume, withdrawn at the average rate of 10 ml. per kgm. per minute. In addition to the blood lost in the hemorrhage, 15 ml. were withdrawn for the control determination of blood volume and 20 to 30 ml. for the blood volume determinations after hemorrhage. Following the acute blood loss, the animals were observed for five to six hours or until death supervened. Observations were made of changes in blood pressure, heart rate, and rectal temperature at frequent intervals. An autopsy was performed at death for gross examination of the viscera. Those dogs which did not die in five or six hours were observed for the next few days until survival was assured.

Plasma volume determinations were made by the dye dilution method using the blue dye T-1824 (Gregersen and Stewart, 1939) and extrapolation of the time-

<sup>1</sup> This work was done partly under a grant from the Josiah Macy, Jr. Foundation and partly under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and Columbia University.

<sup>2</sup> Submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, in the Faculty of Pure Science, Columbia University.

<sup>3</sup> Two parts of methyl methylene para amino phenylformate and five parts of hydrox-benzocarbinoil in refined almond oil. (Caso Laboratories, New York City.)

concentration curve on a semilogarithmic plot (Gregersen and Rawson, 1943). Relative changes in the serum protein concentration were estimated from the changes in the refractive index of the serum by the application of the formula of Neuhausen and Rioch (1923). In a number of experiments these values were checked against protein determinations by the micro-Kjeldahl method. The determinations showed that, although the absolute values in some animals were slightly different ( $\pm 0.25$  gram per cent), there was close agreement between the relative changes in protein concentration as determined by the two methods. The hematocrit was determined on heparinized blood by centrifugation at 3,000 r.p.m. for 30 minutes using the Wintrobe hematocrit tube. The total blood volume was calculated from the measured plasma volume and the hematocrit value by the following formula:

$$\text{Total blood volume} = \frac{\text{Plasma Volume} \times 100}{100 - (0.96 \times \text{Hematocrit})}$$

where 0.96 is the hematocrit correction factor for plasma trapped between the cells (Gregersen and Schiro, 1938).

**RESULTS.** Of the twenty-three dogs, each subjected to a potentially fatal hemorrhage by a single massive bleeding, thirteen died and ten survived. The magnitude of the hemorrhage sustained by the dogs which died averaged 51.9 per cent of the control blood volume (range 44.8 to 58.5 per cent). The average loss for those dogs which survived was 47.6 per cent (range 44.0 to 50.5 per cent). These average figures are equivalent to a loss, in terms of the per cent of body weight, of 5.48 for the group which died and 5.05 for the survivals. The average duration of the post hemorrhage or shock period for those dogs which died was 4 hours, 22 minutes (range 2 hrs., 5 min. to 6 hrs., 30 min.).

The blood volume data for both groups are presented in table 1. The per cent blood volume reduction was calculated from the difference between the control blood volume and the volume measured approximately one hour after hemorrhage. The average per cent reduction in the 13 dogs which died was 43.0 (range 39.1 to 48.0). In the 10 dogs which survived a nearly comparable hemorrhage the average reduction was 34.5 per cent (range 29.0 to 39.7 per cent). The difference between the per cent removed and the per cent reduction is due to the compensatory replacement of the blood volume.

If the replacement is expressed as a percentage of the control blood volume, the results show that the dogs which died had a reserve volume equivalent to 8.9 per cent, whereas those surviving had a reserve of 13.2 per cent. The group which succumbed, therefore, had only two-thirds as large a reserve capacity as the group which survived. These data are graphically presented by the scatter-gram in figure 1.

The reserve capacity may be further analyzed in terms of its two components, i.e., fluid and cells. The dogs which died replaced 9.0 and 9.3 per cent respectively of the control plasma and cell volumes, whereas the corresponding figures for the animals which survived were 11.0 and 17.0 per cent.

These shifts of fluid and cells into the active circulation occurred very rapidly.

The dilution of the serum proteins paralleled the fall in blood pressure and was practically complete by the end of the hemorrhage. The average concentration fell from 6.1 to 5.1 grams per cent in those dogs which died and from 5.9 to 5.0 in

TABLE 1

EXP. NO.	WT.	CONTROL		% BLED		BLED	POST HEMORR.		% RE- DUCTION BV	% RE- PLACE- MENT BV	SUR- VIVAL TIME
		PV I	BV I	PV	BV		PV II	BV II			
13 dogs died in shock											
						cc./kgm.					min
1	8.7	475	915	54.7	58.5	61.6	260	475	48.0	10.5	270
2	13.3	690	1300	54.4	57.0	55.6	425	765	41.2	15.8	225
3	9.2	500	750	51.0	56.6	49.0	260	400	46.6	10.0	280
4	11.4	520	945	47.2	55.0	45.6	305	545	42.3	12.7	125
5	13.0	685	1290	51.8	53.8	53.5	365	770	40.4	13.4	330
6	11.5	675	1300	51.1	51.5	58.3	400	730	43.9	7.6	240
7	13.1	1090	1580	51.8	51.5	62.2	640	855	46.0	6.5	180
8	10.0	645	995	48.9	51.3	51.0	360	575	42.2	9.1	175
9	12.7	570	1075	50.9	50.7	43.0	360	640	40.4	10.3	195
10	11.8	560	1120	50.0	50.5	47.8	340	600	46.5	4.0	265
11	9.4	585	970	45.3	47.4	49.0	360	570	41.2	6.2	360
12	9.9	585	1100	44.4	45.5	50.5	400	670	39.1	6.4	235
13	7.8	390	825	43.6	44.8	47.5	250	480	41.7	3.1	380
Average.....	10.9			49.6	51.8	51.9			43.0	8.9	250
10 dogs survived shock											
14	10.0	550	950	50.0	50.5	48.0	370	600	36.8	13.7	
15	12.2	720	1075	42.4	50.2	44.2	475	735	31.6	18.6	
16	11.0	735	1295	45.6	50.0	58.6	534	780	39.7	10.3	
17	11.2	705	1240	48.3	49.6	55.0	460	810	34.6	15.0	
18	15.3	780	1560	44.2	48.5	49.0	530	1040	33.4	15.1	
19	11.4	670	1125	44.4	47.5	47.0	410	735	34.6	12.9	
20	13.5*	580	1075	44.8	47.0	37.5	390	690	35.8	11.2	
21	9.4	665	970	42.1	45.0	47.9	415	665	31.5	13.5	
22	9.0	540	905	41.6	44.2	44.5	390	645	29.0	15.2	
23	10.2	555	1070	44.1	44.0	46.0	380	665	38.0	6.0	
Average.....	11.3			44.8	47.6	47.8			34.5	13.2	

PV = Plasma volume; BV = Blood volume; % Reduction BV =  $\frac{BV I - BV II}{BV I} \times 100$ ;

% Replacement BV =  $\frac{BV II - (BV I - \text{ml. Bled})}{BV I} \times 100 = \% \text{ Bled} - \% \text{ Reduction}$ ;

% Bled includes volume of hemorrhage + all samples up to BV II.

\* Marked obesity.

the surviving animals. Changes in the hematocrit, however, were unpredictable. In some cases the magnitude of the splenic reflex was such that the post-hemorrhagic hematocrit was higher than the control. In other cases it was the same

or lower. The dogs which died showed a fall in hematocrit from the average control value of 45.6 per cent to 42.0 per cent. In the dogs which survived the hematocrit remained, on the average, practically constant going from 43.9 to 44.4 per cent in spite of the simultaneous addition of tissue fluids as evidenced by the fall in protein concentration.

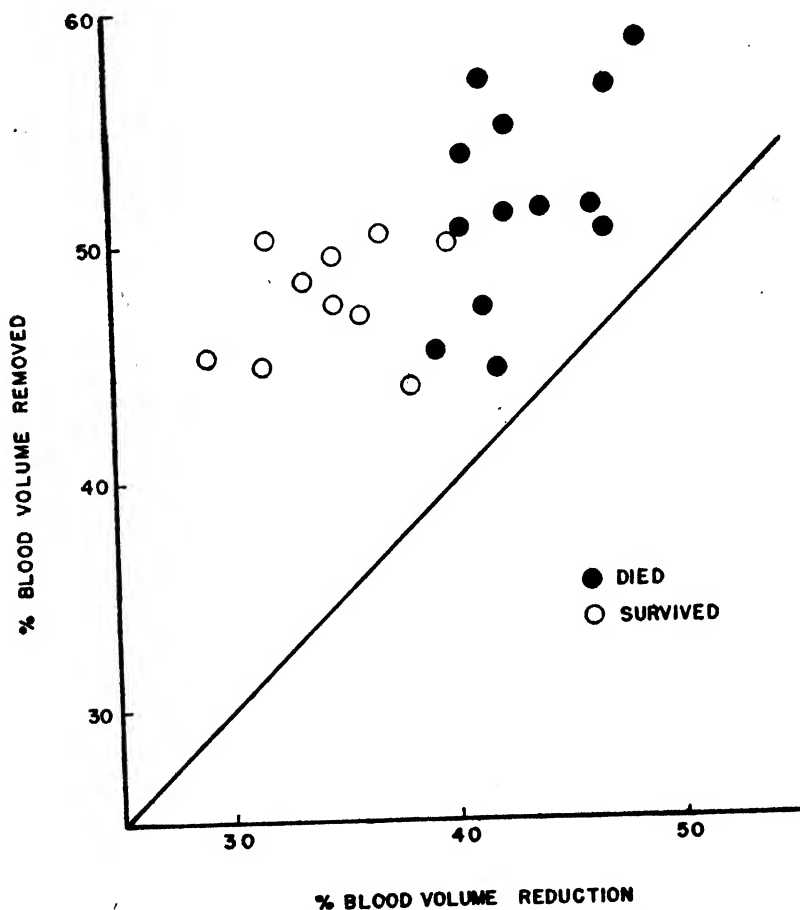


Fig. 1. 23 Dogs bled by single massive hemorrhage

The blood volume determinations were made approximately one hour after the hemorrhage because at this point the conditions were fairly stable. The blood pressure had begun to level off and the hematocrit and protein concentrations remained remarkably constant. Repeated blood volume determinations two to three hours later in six of the surviving animals showed a capacity for continued replacement at the average rate of 2.6 per cent of the control blood volume per hour during the first few hours of the recovery phase. Since the post-hemorrhagic cell volume remained practically constant in these dogs, the cell reserves

having been exhausted, the increase was from the body fluids. This was an increase at the average rate of 6.5 per cent of the control plasma volume per hour. Adolph, Gerbasi and Lepore (1933) reported similar observations on the rapidity of the shifts of cells and fluid in amyralized dogs subjected to rapid, massive blood loss.

Observations on the blood pressure, heart rate, and rectal temperature made at frequent intervals during the post-hemorrhagic period showed the changes characteristic of hemorrhagic shock. The average values for these observations are presented in table 2.

TABLE 2  
*23 dogs bled by single massive hemorrhage*

	13 DIED		10 SURVIVED	
	Aver.	Range	Aver.	Range
Blood pressure (mm. Hg)				
Before hemorrhage.....	113	95-145	105	90-125
5 min. after hem.....	33	20- 45	35	30- 50
Approx. 2 hrs. after hem.....	65	45- 80	70	60 -95
Heart rate (beats/min.)				
Before hemorrhage.....	96	70-135	94	60-130
5 min. after hem.....	126	50-165	132	75-180
Approx. 2 hrs. after hem.....	217	165-280	177	140-200
Rectal temp. (°C.)				
Before hemorrhage.....	39.0	39.8-38.2	39.2	39.5-38.5
Maximum change.....	-1.0	+0.6 to -3.6	-0.2	+1.4 to -1.7

At autopsy, gross examination of the viscera revealed the changes frequently described in fatal hemorrhagic shock. Briefly, the salient findings consisted of submucosal and mucosal hemorrhages in the upper end of the small bowel and, in about half the cases, markedly hemorrhagic areas in the lower colon. The adrenals had flame-like hemorrhages throughout the cortex. Subendocardial hemorrhages of varying degree were always observed in the papillary muscles of the left ventricle and occasionally in the leaflets of the mitral valve.

DISCUSSION. From the quantitative standpoint the study of hemorrhage is a study of the balance between the magnitude of the blood loss and the degree of compensation. There is at one extreme the loss of blood at such a slow rate that the animal's own replacement mechanisms can adequately compensate for the small continuous loss. The other extreme is a rapid exsanguination from which the animal dies immediately. Between these two lies the study of inadequately compensated blood loss which leads to hemorrhagic shock. It is the blood volume at this critical level, or the border between survival and death, with which we are here concerned.

It is well recognized that the volume of blood which it is necessary to withdraw

in order to cause death is a function of the rate of bleeding. This point has been recently studied by Elman et al. (1944). They clearly demonstrated that the magnitude of a fatal hemorrhage is inversely correlated with the volume lost per unit time. The single rapid hemorrhage was employed in the present study in order to prevent, as far as possible, the introduction of the element of time during the bleeding as an additional variable. In order to evaluate the minimum effective blood volume it was necessary to bleed these animals to the point of death. The volume of the hemorrhage, therefore, was of such magnitude that it was potentially fatal. Those animals which died several hours after hemorrhage were considered to have sustained a blood volume reduction slightly in excess of the irreducible minimum. Those animals which survived and were able to recover unaided from the severe hypotension and shock were considered to have been bled to very near the minimum compatible with survival.

The observations on the blood volume reduction in the twenty-three dogs subjected to a single massive hemorrhage may be more clearly discussed by referring to figure 1. The 45° line through the origin is the expected value for all cases. A dog bled 50 per cent of its total blood volume would have an expected reduction of 50 per cent. Because of the mechanisms for compensatory replacement, however, the per cent reduction is always less than the per cent removed. It is apparent that the magnitude of this compensation is the deciding factor (as far as blood volume is concerned) between survival and death in this type of hemorrhage. Those animals which were able to call upon sufficient reserves survived if the blood volume reduction was less than 40 per cent, while those animals which were bled a comparable amount but were unable to restore their depleted volumes as adequately died with a reduction of 40 per cent or greater. This appears to be a rather critical level for the unanesthetized dog. In this series no dog survived a reduction greater than 40 per cent, and only one dog died with a reduction less than 40 per cent. Statistically more significant is the blood volume data on 79 dogs all in hemorrhagic shock but in which shock was produced by different methods of bleeding, i.e., rapid, slow, intermittent. Twenty-nine of these animals survived and only one had a reduction in excess of 40 per cent, while 50 died and only 5 had less than a 40 per cent blood volume reduction at the time when it was measured. Expressed in another way, it would appear that 60 per cent of the normal blood volume is the irreducible minimum compatible with survival for the unanesthetized dog. With less than this amount in circulation the animal will go into progressive shock which, if untreated, results in a fatal outcome within a few hours.

In the course of these experiments, in which shock was produced by a single rapid hemorrhage, five additional dogs were used. The data are not reported here as these animals would have died in less than fifteen minutes had they not received replacement therapy. Two of these dogs were bled 46 per cent of their blood volume and the others between 50 and 56 per cent. This point is discussed in order to emphasize the fact that a standard shock preparation cannot be consistently produced by the letting of a fixed percentage of the measured blood volume. Although from figure 1 it would appear that the removal of 51 per cent

or more of the blood volume would result in 100 per cent fatalities, the scattergram fails to reveal the fact that such a procedure would result in a large percentage of deaths from acute circulatory failure and not hemorrhagic shock.

#### SUMMARY

1. Blood volume studies were carried out on twenty-three unanesthetized dogs in hemorrhagic shock produced by a single rapid bleeding.

2. Determinations were made of the magnitude of the compensatory reserves involved in the early restoration of the blood volume. These reserves averaged 10.7 per cent of the control blood volume but ranged from 3 to 18 per cent.

3. It was concluded that for dogs under these conditions the blood volume must be reduced by at least 40 per cent to lead to progressive fatal shock.

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# STANDARDIZATION OF EXPERIMENTAL HEMORRHAGIC SHOCK<sup>1,2</sup>

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Experimental hemorrhagic shock may be induced either by 1, the rapid removal of a predetermined quantity of blood, or 2, the intermittent letting of a variable quantity until, by one or more criteria, the animal is judged to be in shock. Published methods for the standardization of these procedures are of the second type. Wiggers and Werle (1942) have developed such a method for anesthetized dogs. In principle it is based on the removal at definite time intervals of proportionately smaller volumes of blood until the mean arterial pressure falls to a "shock level." However, in order to avoid the possibility of spontaneous recovery, the mean pressure of all the dogs must be reduced below the level from which any one dog might recover.

Weston et al. (1943) have developed a modification of the graded bleeding method by avoiding the strict adherence to some fixed blood pressure level as the sole criterion of shock. These authors established empirically a set of limiting values for blood pressure, circulation time, and arterial CO<sub>2</sub>. If any two of the three criteria were satisfied, the animal was considered to be in a standard state of shock.

Regardless of the method employed, if the magnitude of the blood loss is limited by the determination of some value which indicates that the state of shock already exists, the period of induction will vary from animal to animal. For this reason it would be advantageous to have a method in which shock could be consistently produced by the rapid removal of some predetermined quantity of blood. This approach would have the additional advantage of permitting observations of the progressive development of shock subject to biological variation instead of forcing the organism to conform to some arbitrary pattern.

Attempts to standardize hemorrhagic shock on the basis of the size of the hemorrhage have, in the past, failed to produce a reliable method. If the bleeding consists of the removal of a fixed quantity of the measured blood volume, the results are uncertain due to the wide range in the dog's ability to compensate for blood loss (Walcott, 1944). If on the other hand the hemorrhage is based on the removal of a fixed quantity of blood per unit of body weight, the results are even more uncertain due to the introduction of an additional variable, i.e., the ratio of blood volume to body weight.

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<sup>2</sup> Submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, in the Faculty of Pure Science, Columbia University.

The solution to the problem lies in the selection of some physiological determinant other than blood volume or body weight. As will be shown in this paper, such a determinant is the bleeding volume. As emphasized by Lawson (1943) this volume is a function of many variables, both constitutional and physiological, such as genetic characteristics, age, the state of hydration and nutrition, the compensatory reserves of both fluid and cells, the total blood volume itself, and the physiological fitness of the circulatory system as a whole. The magnitude of the bleeding volume, then, is the resultant of the combined effects of many of these variables.

Since the bleeding volume is the maximum volume of blood an animal can lose by a single rapid bleeding, it may be defined as a 100 per cent hemorrhage. This offers a convenient basis for standardization. The method in this paper is based on the determination of the bleeding volume and the immediate return of a fixed percentage of the blood collected. The amount returned can be predetermined to produce hemorrhagic shock of varying degree from the mildest symptoms to 100 per cent fatalities. The technique to be described is for the production of fatal shock. It is based on the return of 25 per cent of the bleeding volume and so, for descriptive purposes, may be defined as a 75 per cent hemorrhage.

**TECHNIQUE.** The unanesthetized dog is secured in the supine position and both femoral arteries are exposed, one for taking blood samples and the other for bleeding and recording blood pressure. The arteries are exposed under local procaine hydrochloride which is supported by Neotheson<sup>3</sup> for more lasting local anesthesia. The arterial cannulae, through which the blood is withdrawn, are made of  $\frac{1}{4}$  inch straight glass tubing drawn down to tips of various sizes. The cannula tip is made as stubby and thin-walled as practicable with a minimum amount of "wasting" in order that the effective bore may be as large as possible for any given outside diameter. A piece of "Transflex"<sup>4</sup> transparent plastic tubing  $1\frac{1}{2}$  feet long is attached to the end of the  $2\frac{1}{2}$  inch glass cannula. The bleeding tube and cannula are given a thin coating of petrolatum jelly on the inside, and a drop of Liquaemin<sup>5</sup> is introduced by capillarity into the constricted tip before tying it into the artery. The artery is not tied off and cannulated until just prior to bleeding. These details are presented in order to emphasize the importance of taking every precaution to avoid clotting at any point.

The course of the hemorrhage is brief. The first 200 to 400 ml. of blood are withdrawn, by using slight negative pressure, into 100 ml. syringes moistened with 0.1 ml. of Liquaemin in 1 ml. of saline. The tips of the syringes (without adaptors) are inserted directly into the end of the bleeding tube. The hemorrhage is continued by allowing the blood to run freely into a paraffined graduate from the open end of the bleeding tube held at the level of the heart. Two conditions must be satisfied before the bleeding is terminated: the animal must have

<sup>3</sup> Two parts methyl methylene para amino phenylformate and 5 parts hydroxybenzocarbino in refined almond oil. Caso Laboratories, New York City.

<sup>4</sup> Irvington Varnish and Insulator Co., Irvington, New Jersey.

<sup>5</sup> Heparin in saline. Roche-Organon, Inc., Nutley, New Jersey.

gone through a state of functional decerebration, and the bleeding must be continued for at least 30 seconds after the appearance of an interrupted flow or dripping. This is a necessary precaution as the flow may become interrupted at the first appearance of functional decerebration and later return to a continuous flow if respiration is still active and forceful. When it is apparent that the bleeding is virtually complete, 25 per cent of the blood removed is immediately returned to the circulation. The blood is injected from the 100 ml. syringes as rapidly as possible through a large needle inserted in the femoral vein or, more simply, via the bleeding tube and arterial cannula (see Kohlstaedt and Page, 1943, on the relative merits of the venous and arterial routes for infusions). Frequently the end point of the bleeding coincides with respiratory failure, but as arterial blood is being injected respiration is resumed spontaneously after the

TABLE 1

EXP. NO.	WT.	BV I	BLDG. V.	RETURN	BLDG. V. % BV I	BLDG. V.	BV II	TIME MEAS.	% REDUC. AT 150 MIN.	SUR- VIVAL TIME
	kgm.	ml.	ml.	ml.		m./kg.	ml.	min.		min.
1	13.0	1040	790	195	76.0	60.7	620	190	39.7	345
2	18.6	1675	1300	320	77.6	69.9	990	60	42.6	315
3	11.4	1065	710	170	66.6	62.3	645	80	42.3	310
4	13.4*	1180	940	225	79.6	70.2	665	165	43.6	270
5	9.1	870	540	130	62.0	60.0	485	190	43.1	255
6	14.9	1600	1010	250	63.1	67.8	935	90	43.8	250
7	13.3	1305	820	200	62.8	61.6	720	200	44.2	230
8	11.8	1105	800	195	72.4	67.8	595	130	47.0	200
9	19.8*	1410	1135	275	80.5	57.3	740	130	47.5	183
10	13.2	1200	795	195	66.2	60.2	650	115	47.5	155
Average ...	13.9				70.7	63.8			44.1	250

BV = Blood volume; Bldg. V. = Bleeding volume; % Reduc. at 150 min. =  $[(BV I - (BV II \pm \text{ml. removed between time measured \& 150 min.)}] \times 100$

BV I

\* Marked obesity

re-admission of the first 100 ml. In the present series approximately 10 ml. of blood were withdrawn at half-hour intervals after the hemorrhage for analysis of the changes in blood chemistry and blood volume.

**RESULTS:** Observations have been made on 30 unanesthetized dogs in which hemorrhagic shock was produced by the method described above. All 30 animals died within  $1\frac{1}{4}$  to  $5\frac{1}{2}$  hours, the average survival time being 3 hours, 45 minutes. The bleeding volumes averaged 62.4 ml. per kgm. of body weight (range 42.0 to 88.6 ml. per kgm.). Blood volume determinations on 10 of these dogs were made before and 1 to 3 hours after hemorrhage using the T-1824 dye method (Gregersen and Stewart, 1938; Gregersen and Rawson, 1943). These data are presented in table 1. The bleeding volume averaged 70.7 per cent of the measured blood volume (range 62.0 to 80.5 per cent). The post hemorrhage

determination showed an average blood volume reduction of 45.3 per cent at the time it was measured (range 40.4 to 47.5). The values presented in the table for the per cent reduction have all been corrected to 150 minutes after hemorrhage by adding or subtracting the amount of blood removed as samples between the time of the blood volume determinations and 150 minutes.

A general picture of the blood volume changes is graphically presented in figure 1. This is based on the average values for 10 dogs. At zero time the measured circulating blood volume was considered as 100 per cent. Below the base line the average reserves are represented as equivalent to 14 per cent of the control volume, but individual values varied from 6.9 to 24.0 per cent. This represents

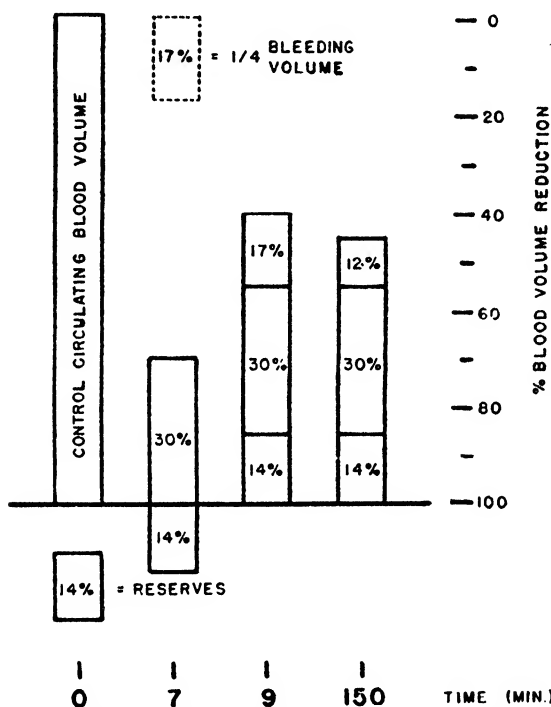


Fig. 1. Blood volume changes 75 per cent hemorrhage 10 dogs (aver.)

the capacity of the animals to mobilize cell reserves and tissue fluids in compensation for blood loss. The determination of the bleeding volume, which required on the average 7 minutes, resulted in the removal of approximately 70 per cent of the blood volume. One quarter of this bleeding volume (equal to 17 per cent of the control volume) was immediately reinjected. The entire procedure was completed in 9 minutes. The reserves are depicted as having moved into the active circulation by the end of the 75 per cent hemorrhage. Although in this series these reserves were not measured until some time later, it has been previously established (Walcott, 1944) that the shift is virtually complete by the end of a rapid massive hemorrhage. The final column represents the blood vol-

ume approximately 150 minutes after hemorrhage showing the further depletion caused by the removal of half-hour blood samples. These 10 dogs had at this time an average blood volume reduction of 44 per cent.

The clinical course of these animals after the acute blood loss followed a pattern typical of the unanesthetized dog in fatal hemorrhagic shock. From an initial hypotension of approximately 40 mm. Hg the blood pressure recovered to an average value of 60 mm. Hg and remained at this plateau until the last third of the survival period during which it slowly fell off to 25 mm. Hg and then rapidly to zero. The heart rate, however, continued to increase as shock pro-

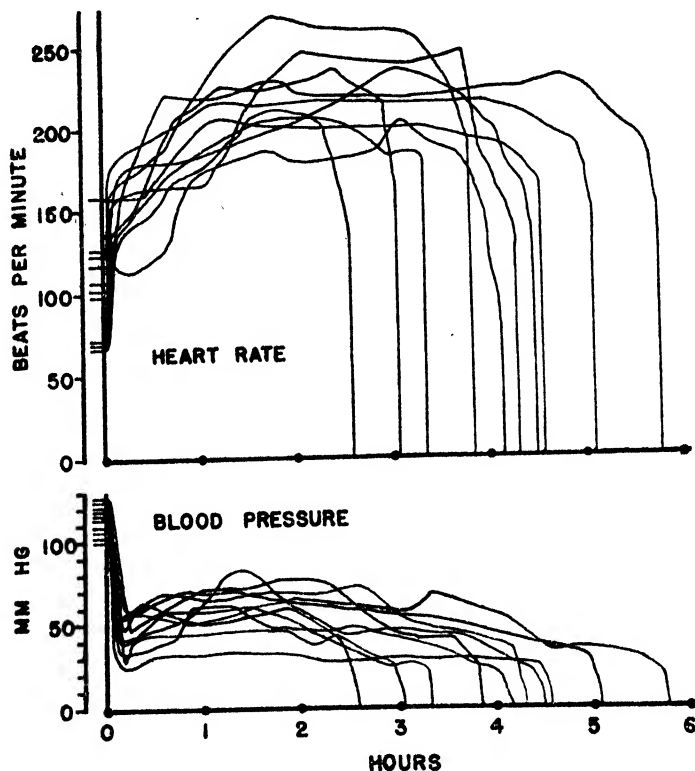


Fig. 2. 75 % hemorrhage 10 dogs

gressed, rapidly at first and then more slowly, reaching an average maximum of 214 beats per minute one-half hour before death. The charts of blood pressure and heart rate for 10 of these dogs have been reproduced in figure 2. Respiration invariably failed before the heart stopped beating. During the gradual decline of blood pressure in the late stages of shock, the animals showed signs of restlessness passing over into a state of depression and finally coma. During the initial and terminal periods of hypotension, the animals usually went through the motions characteristic of the act of defecation. These motions were repeated at short intervals and, although they soon ceased to be expulsive, they were often continued for long periods.

Autopsies were performed at death on each animal for gross examination of the viscera. The pathological changes in the heart and intestinal mucosa associated with hemorrhagic shock in the dog were invariably observed. These changes will not be dealt with in this paper. It may be interesting to note, however, that the extent of the pathology of the lower bowel was directly correlated with the defecation motions. Those animals in which the motions occurred at more frequent intervals and for longer periods of time showed the most marked mucosal and submucosal hemorrhages of the sigmoid colon and rectum.

**DISCUSSION.** The question as to whether the procedure of determining the bleeding volume does not in itself predispose to fatal shock may be answered by a consideration of the following evidence. Blood volume measurements on one dog subjected to the determination of its bleeding volume, but in which all the blood was immediately returned, showed a loss one hour later of only 5 ml. over and above the 15 ml. removed as samples. The other criteria remained virtually unchanged except for a slight and transient elevation of the blood pressure immediately after the reinfusion. This might be ascribed to a persistence of the sympathetic discharge and the temporary augmentation of the blood volume by the influx of tissue fluids and cell reserves during the bleeding. An additional 5 dogs were subjected to a 50 per cent hemorrhage (50 per cent of the bleeding volume returned). The post-hemorrhagic blood volume was measured in one of these dogs. The reduction was 23.2 per cent. All 5 dogs showed only a moderate degree of hypotension. One hour after bleeding the mean arterial pressure averaged 88 mm. Hg and the heart rate 144 beats per minute. They all survived and were observed during the recovery period for 5 days before being sacrificed.

Another group of 5 dogs was subjected to a 63 per cent hemorrhage (37 per cent of the bleeding volume returned). Four of these animals survived with an average blood volume reduction of 30.7 per cent. The blood pressure one hour after bleeding averaged 61 mm. Hg and the heart rate 130 beats per minute. Blood chemistry studies of lactate, phosphate, blood gases, and pH showed evidence of moderately severe, but not progressive, hemorrhagic shock. These 4 dogs, although subjected to a continued blood loss of 10 ml. every half-hour for 5 hours after bleeding and kept in the cage for an additional 16 hours without food or water, showed a capacity for continuous replacement of the plasma volume. The dogs were sacrificed on the fifth day. The fifth dog in this group which underwent a 63 per cent hemorrhage died in the cage some 10 hours after bleeding. It was apparent from the control blood volume that this animal came to experimentation in a dehydrated state. One hour after hemorrhage the blood volume reduction was 36.4 per cent. The volume of blood samples removed during the subsequent 4 hours of experimentation was sufficient to cause a calculated reduction of 42.5 per cent. This is in excess of the critical level of 40 per cent reduction (Walcott, 1944) and would explain the fatal outcome.

The observation that unanesthetized dogs which die in hemorrhagic shock have a blood volume reduction of 40 per cent or greater is further substantiated by the data from the present series. The 10 dogs in which the blood volume was determined 1 to 3 hours after a 75 per cent hemorrhage had an average reduction

at that time of 45.3 per cent. The range from 40.4 to 47.5 per cent is probably caused mainly by variations in the capacity for replacement at the time of hemorrhage. In general those dogs with the greatest reserves had the least reduction after the bleeding.

The duration of the survival period is inversely related to the extent of the blood volume reduction. That the animals with the greatest reduction died in the shortest period of time is shown in figure 3. The implications of this observation may be further discussed. It is possible to calculate the terminal blood volume reduction for each animal by subtracting from the measured post hemorrhage volume the blood samples subsequently removed up to the time of death. This predicted terminal reduction averaged 48 per cent with a range of only  $\pm 1.5$  per cent. It would appear then that the procedure of withdrawing blood samples every half hour is an essential part of the method where a 75 per cent hemorrhage is employed. It is as though the animal would live until the blood volume had been reduced to 52 per cent of the normal—a terminal value incompatible with life. Had the samples not been withdrawn, the survival time of some of these animals would undoubtedly have been prolonged, perhaps even to the point of recovery.

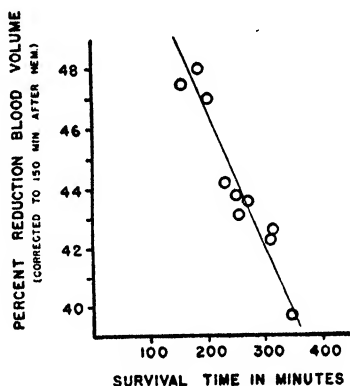


Fig. 3. 75 % hemorrhage 10 dogs

It is more than a presumption that the removal of the bleeding volume is a fatal procedure if none is replaced. The decerebrate rigidity, the frequent failure of the respiratory center, and the fact that the blood was dripping very slowly all point to a totally inadequate circulation. In fact, all 30 dogs died despite the 25 per cent replacement of which less than half was subsequently removed as samples. This 100 per cent mortality is difficult to reconcile with the results of the experiments reported by Ivy et al. (1943), who used the bleeding volume in a study of blood substitutes in unanesthetized dogs. In their control series 8 out of 50 dogs survived the determination of the bleeding volume without any subsequent replacement. A recalculation of their published data indicates an average bleeding volume of 51.5 ml. per kilogram, with the animals having a bleeding volume less than 36 ml. per kilogram discarded from the series. The average

bleeding volume of the 30 dogs reported in the present paper was 62 ml. per kilogram. The lowest value recorded was 42 ml. per kilogram in a dog almost pathologically fat; the next lowest value was 51.2 ml. per kilogram. The difference between the higher bleeding volumes reported in this paper and the results of Ivy et al. might be due to the recognition that the flow may resume after it first becomes interrupted. Continued bleeding to the end point, which is assured by the strictest avoidance of all possibilities for clotting in the bleeding apparatus, is the most probable explanation for the higher bleeding volumes found by the present writer.

The values for the bleeding volumes reported in this paper are considerably higher than those obtained under various anesthetics. When ether was used as the anesthetic agent, Mann (1915) and Roome, Keith and Phemister (1933) found arterial bleeding volumes of 48.2 ml. per kilogram and 40.0 ml. per kilogram respectively. The latter authors also reported that the bleeding volume of dogs under barbital anesthesia was 45.5 ml. per kilogram. Harkins (1935) used barbital and found a value of 39.0 ml. per kilogram.

#### SUMMARY

1. A method is presented for the standardization of hemorrhagic shock in the unanesthetized dog.

2. The method consists of the rapid determination of the bleeding volume followed immediately by infusion of 25 per cent of the blood collected. This procedure requires less than 10 minutes. A fixed volume of 10 ml. is subsequently withdrawn at half-hour intervals.

3. This method of bleeding invariably produces fatal hemorrhagic shock.

4. In a series of 30 dogs on which this method was employed the survival time averaged 3 hours, 45 minutes.

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# STATISTICALLY VALID TESTS OF DRUGS AND OTHER FACTORS AFFECTING THE RESISTANCE OF MICE TO ACCELERATION<sup>1</sup>

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The effect on man, in the seated posture, of acceleration in the same direction as that of gravity, so that he is pushed harder into his seat, is of great interest because military aviators, in combat maneuvers, are subjected to this type of stress. The many advantages of using small experimental animals in the exploratory phases of this problem are off-set, to some degree, by certain disadvantages, which should be explicitly weighed when animal experimentation is performed with the human problem as the main consideration.

Man is a biped and so has developed a vascular system peculiarly suited to react quickly and favorably to the sudden assumption of the upright position. Experiments on animals other than the primates are therefore subject to the criticism that the inherent mechanisms to withstand acceleration in these animals are probably not equivalent to man's. For this reason, results drawn from such animals must apply with less cogency to man than is generally the case. But they can nonetheless serve as guideposts in the choice of human experiments.

Another difficulty in experiments on smaller animals is that the practical convenience of choosing death as the end-point of the biometric titration means that we compare death in the animal with "black-out" (retinal ischemia) in man. This difficulty, while real, does not seem intrinsically invalidating, since there must be some inherent relationship (subject to biological variation) between the "black-out" threshold and the acceleration required to cause death.

In man, the acceleration is applied for a short duration, usually not more than 20 seconds, and the time-acceleration curve is not symmetrical. How should one duplicate this physiological event in a mouse, for example? Should one choose the same time span for acceleration as in man? Or should the span be adjusted to the relative life spans of the two species; or to the relative duration of a single normal respiration or heart beat? This quandary is a real one because resistance to acceleration in man appears to be composed of instantaneous resistance—primarily the ability of the brain to maintain function ischemically, and subsequent resistance, secondary to the ability of the vasomotor system to restore blood to the brain. (Still later, other mechanisms enter.) None of the

<sup>1</sup> The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the John B. Pierce Foundation.

<sup>2</sup> Now Lieutenant Commander, MC-(S), USNR. The opinions or assertions contained herein are the private ones of the authors and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large.

previously mentioned hypotheses for choosing a time of exposure to acceleration calls for a longer acceleration span than in man, yet many of the animal experiments hitherto reported have used such intervals. Practically, one is constrained to use a minimal duration roughly equal (though perhaps not biologically) to a 20 second exposure in man, because the high acceleration required for a lethal result in small animals cannot be achieved faster without a large special centrifuge, or an untowardly high angular acceleration, itself a complicating factor.

A consequence of the choice of death as the end-point is that the control for each treated animal must, of course, be another animal, rather than himself, as is the case in man. This result, however, is not of great moment, since statistical analysis on adequately large numbers can reveal validity, irrespective of this necessity for the use of a separate control group.

The value of experimentation with the mouse or rat, even though the conclusions cannot be translated into terms for humans without reservations and subsequent confirmation, is considerable. The small animals are very inexpensive, compared to the pay for human subjects; they are still easily available, unlike young healthy adult male humans today; they require almost no expenditure of money for apparatus, as compared to the high cost of the human centrifuge; and they take much less time than is needed in experiments on man. Many of the difficulties in using small animals, as has been noted above, can be minimized or eliminated by proper design of statistically valid experiments. Examples of experiments on mice, using the ordinary laboratory electric centrifuge, will be given in which the effects of harmful, innocuous and beneficial drugs and of the mouse's weight and previous posture are illustrated.

**METHOD.** The laboratory electric centrifuge with rheostat was first chosen, since it is generally available. The mouse was thereupon decided on as the experimental animal, since it will fit comfortably into the 50 cc. centrifuge cup, which provides it with a centrifuge of approximately an 8 inch radius. The ratio of this radius to the length of the mouse compares favorably with the corresponding ratio of the best human centrifuge.

All the mice were drawn from pure, thoroughly stabilized, albino colonies. Two separate colonies were utilized, but in the statistical analysis the two groups were also treated quite separately so that homogeneity of the biological material was as great as possible. Only young adult males were used, since the interest is primarily in military applications to young men.

The mice were placed in cylindrical wire mesh cages, open at one end, about the size of the 50 cc. glass centrifuge tube. The cage was then placed in the brass cup so that the closed end of the cage protruded slightly, and the mouse would stay upright, until swung, so that the force of the acceleration was always caudally directed. The animals were somewhat supported by the cage and cup in a crouched position similar to sitting, once the acceleration started, so that hernia of the abdominal wall rarely occurred.

The program of an experiment was to place the mice in the cages, the treated one always diametrically opposite his control, and then, after making sure that

they all had remained head up, they were spun by rapidly moving the rheostat pointer to no. 5 on its dial (an arbitrary position). After 15 seconds at this position, with the centrifuge steadily gathering speed, the electric current was interrupted, allowing the centrifuge to coast for  $2\frac{1}{2}$  seconds, and then the brake was applied, stopping the centrifuge in the remaining  $2\frac{1}{2}$  seconds, to complete a run of 20 seconds' duration. The mice were now examined promptly to make sure none were dead or *in extremis*. Peering through the mesh cage or blowing on them usually sufficed to answer this question, but in case of any doubt the cage was withdrawn from the cup. A mouse either recovers rapidly or dies soon after being spun, in our experience, so that little trouble was experienced in determining the result of a spin. When a mouse died, it was left in the centrifuge to maintain the weight and balance of the rotor, while the number of the spin in which the mouse died was recorded on a piece of paper placed in his cage.

After the examination was completed—in no case less than one minute, the centrifuge was spun again for the second time, in every way like the first spin, except that the rheostat pointer was advanced to no. 6 instead of no. 5. Successive spins went to successively higher numbers on the rheostat dial (higher speeds), the experiment being terminated when all the animals had been killed. Then their weights and their fatal spin numbers (the number of successively faster spin trials required to kill them, FSN) were recorded with those of their controls.

While the weights of the mice were restricted by the choice of young adult males, no further attempt was made to pair animals by weight. Examination of figure 1 in which the average weights of mice for each fatal spin number (FSN) throughout the range are compared, shows no correlation between weight and acceleration resistance, which justifies a completely indiscriminate pairing.

Control and test mouse were always treated exactly alike. When drugs were tested, they were administered intraperitoneally, dissolved in saline, compared with a pure saline control, and the skin puncture was sealed immediately with Duco cement.

To test the effect of previous posture on the resistance of mice to acceleration, they were confined in cylindrical cages with access to water and food. The control group was confined but maintained horizontal and the test group was tilted at an angle of  $45^\circ$  or  $30^\circ$ . In the first study, the cages were wide enough to permit the mice to turn around with difficulty. Casual observation indicated, surprisingly, no apparent predilection for either head-up or head-down tilting. The later experiments therefore maintained all the mice in cages so narrow as to prevent their turning around.

**STATISTICAL METHODS.** In the absence of knowledge that the fatal spin numbers (FSN) of our strain of mice were normally distributed about some central value, it was unjustified to take FSN as an index of acceleration resistance, average the results for many mice, and compare, with the usual statistical methods, this average with the average for a control group, even assuming that runs for the centrifuge at the various rheostat settings were adequately repro-

ducible. If FSN were not distributed in accordance with the probability curve, the usual statistical methods, which assume such a normal distribution, would not apply. Accordingly, we had the alternative of first demonstrating the normal distribution of FSN or of using a statistical method not dependent on the assumption of normality. Fortunately, the second alternative did not exclude the first: The method chosen ultimately permitted a fairly satisfactory concomitant demonstration of the validity of FSN as an index of acceleration resistance, without the risk of loss of mice and time if FSN had turned out not to be normally distributed.

Of the three possibilities, namely, that the treated mouse is more resistant, less resistant, or equal in acceleration resistance to his control, only the first two are statistically useful, although the frequency of occurrence of pairs of equal resistance has value in weighing the relative significance of results. Discarding pairs in which both mice died on the same spin, one is left with a set of pairs where the treated animal died after his control, and a set where he died before his control, corresponding to heads and tails in coin-tossing. Here it is not necessary for the statistical analysis for the centrifuge runs to be very similar (although they were, so far as possible, since we wished to use FSN as an index), because the treated mouse is compared solely with his control. It is important that similarity be maintained only so that the same type of acceleration resistance in different pairs is studied. Accordingly, the usual binomial coefficient expansion, as used in the analysis of the probability in coin-tossing, becomes applicable. Tables 1 to 3 illustrate this mode of analysis.

Meanwhile, as these data accumulated, the FSN of the controls themselves could be made into a table to determine whether FSN were normally distributed. Inspection of the general shape of the envelope of the columns shown in figure 1 indicated that FSN was fairly normally distributed and that it could properly be used statistically as a numerical index of acceleration resistance.

One plan of experiment, using FSN, is to confine one's attention to the indicated acceleration protection in terms of FSN of each experimental pair. For example, if an experimental animal died on the sixth spin and his control died on the seventh spin, the protection conferred would be minus 1; if the control died on the fourth spin, protection would be plus 2. In this way, if 15 pairs of mice were studied, 15 values of protection would be found, and one can then determine whether the average protection afforded (whether positive or negative) is significantly different from zero. However, alternatively, one could average separately the FSN of the 15 controls and of the 15 treated mice and then determine whether the difference between the means is significant: that is, whether the experimental and the control groups can still be considered interchangeable selections from the same untreated population of mice. This plan has the great virtue that, where small numbers are involved, and the "students" *t* method is employed, in obtaining conclusions from small numbers (less than 30), the table for *t* is entered for two less than the *total* number of animals (28, in this example), instead of one less than the number of *pairs* (14, in this example) (1). Such a

method, while it increases the effective number of animals, increases the precision of discrimination of a positive from a negative result only if the homogeneity of the mice and the uniformity of the experimental procedure are adequate to render unimportant the matching statistically of each treated mouse by his own control. When we examined our data by these two methods, we found that the more economical one, where matching was not the basis, could be employed, and we have therefore used it.

RESULTS. 1. *Weight*: Figure 1 has already been referred to above. The average weight of the mice dying at each spin number is seen to be unrelated to the spin number. We conclude that the weight of our young adult male mice did not significantly affect their relationship to acceleration.

Number of Mice (Each Weight Recorded)	20					18.1	14.6	15.1				
						16.0	11.5	16.5				
15						16.5	14.8	12.5				
					13.3	10.0	17.4	11.6				
					15.4	10.0	19.5	10.6				
					13.3	15.2	13.9	13.5				
10				14.4	12.2	16.8	18.0	18.0				
				10.8	20.5	17.1	17.8	18.9				
				11.0	14.9	13.2	18.3	19.3	15.5			
				13.6	17.1	25.4	14.5	17.0	11.5	13.5		
				15.7	16.5	17.2	16.2	16.5	14.7	16.1		
5				17.1	18.4	17.9	16.6	13.4	15.2	16.7		
				16.6	16.3	19.0	16.2	15.2	20.6	18.5		
				17.9	17.5	18.7	16.9	17.5	15.5	17.6		
				16.7	17.7	16.6	15.0	17.4	16.4	16.8		
				13.2	17.0	18.0	16.5	18.0	15.6	16.6	15.6	17.8
		15.7		13.5	18.7	16.0	17.5	13.0	17.5	19.0	17.0	14.6
Fatal spin number.....	1	2	3	4	5	6	7	8	9	10	11	12
Average weight.....		15.7		14.6	16.3	16.6	16.2	15.5	15.8	16.8	16.3	16.2

Fig. 1. Fairly normal statistical distribution of fatal spin numbers justifying their use as index of acceleration resistance.

Lack of correlation between acceleration resistance and weight in 98 young adult male mice.

2. *Ergonovine*: One of the alkaloids found in the ergot group is ergonovine.<sup>3</sup> It is supposed to stimulate the sympathetic nervous system without antagonizing adrenalin (2). Its effect on mice was tested with the results seen in table 1, where the "coin-tossing" type of statistical analysis described was employed.

It is apparent that large doses are definitely deleterious and so, to a lesser extent, are the smaller doses. It is clear from the table that at no range of dos-

<sup>3</sup> We are indebted to Dr. K. K. Chen and Eli Lilly & Company for a generous supply of this drug.

age was there evidence of a beneficial effect of the drug. Slight exophthalmos, piloerection, and unsteady gait were found after toxic doses.

TABLE 1  
*The effect of ergonovine (Lilly)*  
Deleterious in large doses. Protection by smaller doses

DOSE ERGONOVINE  mgm.	NUMBER OF TREATED MICE				PER CENT LIKE- LIHOOD OF RESULT BEING DUE TO CHANCE
	Total treated	Same resistance as control	More resistance than control	Less resistance than control	
0.4	19	2	2	15	0.1
0.2	17	4	5	8	29
0.1	15	2	4	9	13
0.025	18	6	5	7	39
0.2	50	12	14	24	7
0.1					
0.025					
0.00625	21	1	11	9	41

3. *Pitressin*: Pitressin is believed to improve acceleration resistance in man (3). The same "coin-tossing" method of analysis is used here as for ergonovine.

TABLE 2  
*The effect of pitressin*  
Confers protection on low resistant mice alone

	NUMBER OF TREATED MICE—PITRESSIN				PER CENT LIKELIHOOD OF RESULT BEING DUE TO CHANCE
	Total	Same resistance as control	More resistance than control	Less resistance than control	
Death of one of pair before 7th spin....	12	1	11	0	0.05
Death of one of pair after 6th spin.....	29	4	14	11	35
Total.....	41	5	25	11	1.40

While pitressin clearly is statistically effective, when the group is examined as a whole, it is noteworthy that its influence is primarily in the least resistant third of the mice.

TABLE 3  
*The effect of pitressin with atropine*  
Greater protection than with pitressin alone

NUMBER OF TREATED MICE—PITRESSIN AND ATROPINE				PER CENT LIKELIHOOD OF RESULT BEING DUE TO CHANCE
Total	Same resistance as control	More resistance than control	Less resistance than control	
20	1	16	3	0.2

4. *Pitressin with atropine*: When atropine is added to the pitressin, the net

effect is more marked than that of pitressin alone, as seen in table 3, where again the "coin-tossing" analysis is utilized.

The addition of atropine has increased the resistance of the whole group of mice, rather than only the least resistant one-third, since that group, as shown in table 2, had already received enough protection so that none of the treated mice remained in this group.

In table 4, the result of numerical FSN analysis, averaging the control and treated groups separately, is shown. Pitressin with atropine increased the FSN by 1.90 (from 7.85 to 9.75) with only a 3/10 per cent likelihood that this result is due to a chance error.

A graphic illustration of the statistical method used is given in figure 2.

TABLE 4  
*Numerical measurement of resistance conferred by pitressin with atropine*

NUMBER OF MICE	MEAN RESISTANCE (FATAL SPIN NUMBER)	STANDARD ERROR OF MEAN RESISTANCE	MEAN PROTECTION CONFERRED	STANDARD ERROR OF MEAN PROTECTION CONFERRED	PER CENT LIKELIHOOD OF RESULT BEING DUE TO CHANCE
Control.....	7.85	0.49			
Pitressin with atropine.....	9.75	0.39	1.90	0.63	0.3

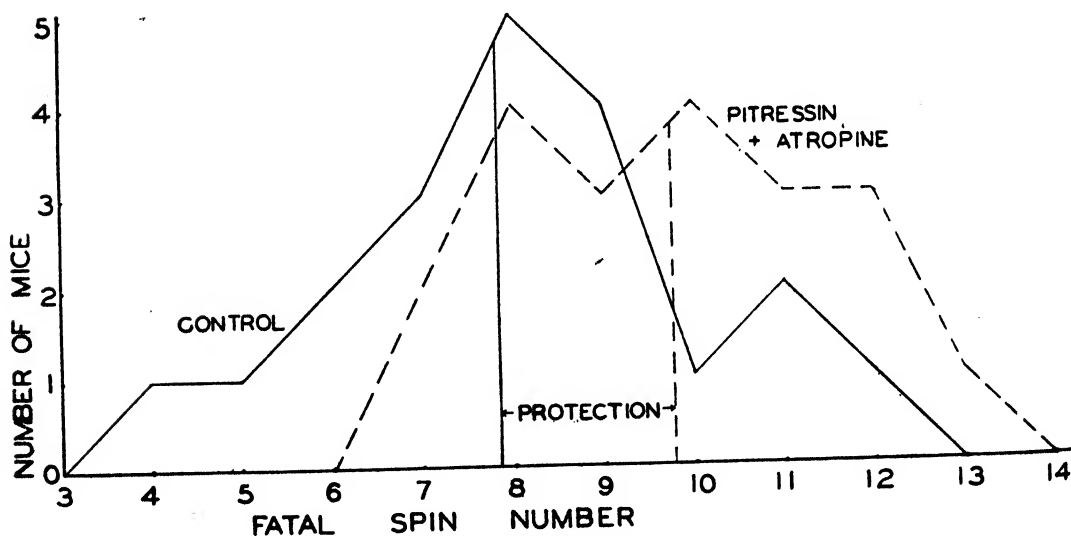


Fig. 2. Protection by pitressin and atropine

5. *Dilantin*: No significant effect of dilantin was found. In 14 cases, the treated mouse outlived his control; in 11 cases, he died first; in 2 cases they died together. Accordingly, the drug is considered innocuous insofar as resistance to acceleration is concerned.

6. *Previous posture*: The symptoms of orthostatic hypotension have been suc-

cessfully treated by having the patients sleep in beds tilted slightly, head up (4). The possibility that keeping mice continuously tilted before centrifuging might raise their resistance to acceleration was therefore explored by confining them in cylindrical wire-mesh tubes supplied with food and water. Half of the cages were tilted at 45° with the mice head up. However, the mice did not maintain this position: at any time, about half would be found head down.

TABLE 5  
*Effect of inclined posture for one week*  
No protection found

NUMBER OF TILTED MICE				MEAN PROTECTION IN FATAL SPIN NO. (FSN)	PER CENT LIKE- LIHOOD OF RESULT BEING DUE TO CHANCE
Total	Same resistance as control	More resistance than control	Less resistance than control		
13	2	6	5	0.3	50

It was found that all but one of the mice (a control) survived and that the tilted posture without regard to the position of the head had no significant effect on acceleration resistance.

Since the mice did not prefer the head-up position, they were confined in narrower cages in which they could not turn around. Problems of feeding and watering were somewhat difficult. Six of the fourteen mice tilted to 45° died in the 4-day period of the experiment, as did 2 of the 14 confined controls. The 8 remaining tilted mice were spun, as were the 12 controls, the method of group analysis previously described being employed so that all 16 mice could be counted.

TABLE 6  
*Effect of semi-upright posture for four days*

NUMBER OF TILTED MICE				MEAN PROTECTION IN FATAL SPIN NO. (FSN)	PER CENT LIKE- LIHOOD OF RESULT BEING DUE TO CHANCE
Total	Same resistance as control	More resistance than control	Less resistance than control		
8	0	7	1	2.1	3.5 6.4

The tilted survivors were found to be significantly more resistant than the controls, with an estimated protection of 2.1 FSN.

However, this result is complicated by the fact that almost half of the tilted group were eliminated, possibly because their resistance to the acceleration of gravity was lower than that of the survivors. That is why this experiment does not yield an unambiguous result.

Repetition of the experiment, with completely adequate methods of feeding and watering, using an angle of 30° tilt for 2 days (this period at a very much smaller angle applied at night is efficacious in orthostatic hypotension in man),



the period which all of the mice survived in the experiment given in table 6, gave the following result:

TABLE 7  
*Effect of semi-upright posture for two days*  
No significant effect

NUMBER OF TILTED MICE				MEAN PROTECTION IN FATAL SPIN NO. (FSN)	PER CENT LIKE- LIHOOD OF RESULT BEING DUE TO CHANCE
Total	Same resistance as control	More resistance than control	Less resistance than control		
14	2	8	4	1.14	19 20-30

An average protection of 1.14 FSN was found, which is not significant.

DISCUSSION. While acceleration resistance is commonly considered to be dependent, to some extent, on age, within our range of young adult mice no correlation was found.

Why ergonovine reduced the acceleration threshold of our mice is not clear. This drug has not been widely studied so that conclusions concerning its expected activity are subject to considerable error.

The effect of pitressin is of considerable interest, since the analysis indicated its value only in the least resistant mice, where its effect is so considerable as still to be revealed even when the whole group is studied, despite the dilution of one-third by the more resistant unaffected two-thirds of the group. The vagus slowing of the heart caused by pitressin can be abolished by atropine, thus causing a sustained increase in blood pressure (5). It seems likely that the beneficial effect of pitressin with atropine<sup>4</sup> found in the whole group may be explained by the sustained increase in blood pressure, caused by the combination of these drugs, which tends to counteract the increase in the normal pressure decrement between brain arteries and aorta found during radial acceleration. However, this conclusion is tentative, since the effect of atropine alone was not assayed.

The innocuous effect of dilantin suggests that the value of this drug in raising the altitude ceiling of mice (6) is restricted to cerebral anoxia and does not extend to cerebral ischemia, the probable cause of death in our accelerated mice.

The results of tilting mice preliminary to centrifuging them are difficult to interpret. Though there was slight protection found on the average (1.14 FSN), this result with the numbers employed was not significant. Possibly, repetition of the experiment in sufficient numbers would confirm the result and render it significant. Possibly, more than a two day period of tilting is needed to reveal a more definite result. However, in line with our earlier caution as to the translation of results of experiments on mice to men, even a negative result in mice should not militate against trial of the tilted bed procedure in man, like that successful in hypotension, since the vasomotor system of a biped is so much more likely to be highly capable of adapting itself to changes in position than that of a quadruped.

<sup>4</sup> Atropinization was sufficient to increase pulse rate.

Our main purpose here has been to illustrate a technique for obtaining statistically valid conclusions concerning acceleration resistance of mice on the ordinary laboratory centrifuge. Examples of deleterious, innocuous, and beneficial drugs have been recorded, as well as the effects of another type of procedure—preliminary tilting.

#### SUMMARY AND CONCLUSIONS

1. Mice serve as satisfactory animals in the laboratory centrifuge for measuring numerically resistance to acceleration in terms of fatal spin number (FSN), the number of spins of equal duration, but progressively higher top speed, required to cause death.

2. Ergonovine, throughout its dose range, reduces the resistance of mice to acceleration.

3. Pitressin increases the resistance to acceleration of mice with low initial resistance and is without influence on mice of average or high resistance.

4. Pitressin with atropine extends the protection to acceleration afforded mice by pitressin alone. The protection of the combination is 1.9 FSN.

5. Dilantin is without effect on the acceleration resistance of mice.

6. Preliminary tilting of mice, without maintaining the head up, has no effect on the acceleration resistance of mice. Tilting, keeping the head up, may possibly have a protective influence if continued for 4 days; after 2 days, the slight beneficial influence found was not significant.

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# STANDARD HUMAN SERUM ALBUMIN STABILIZED WITH SODIUM PHENYLACETATE AND SODIUM MANDELATE

## TOXICITY STUDIES

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Observations of Ballou, Boyer, Luck and Lum (1944a, b) have shown that the thermal stability of standard human serum albumin solutions is significantly increased by the addition of non-polar anions in low concentration. Nephelometer studies and "cloud-point" determinations by these authors demonstrated that such anions enhanced the stability of standard 25 per cent human serum albumin at 50° and 57° and raised the 30 second "cloud-point" temperature. The following substances, sodium butyrate, sodium caproate, sodium caprylate, sodium phenylacetate, sodium mandelate and sodium phenylbutyrate, all were found to be effective stabilizers of the albumin solution. The possibility of stabilizing albumin to higher temperatures and for longer times than is now feasible has prompted an investigation of the hemolytic effects and toxicity of such stabilizing agents.

From a comparison of the results of nephelometric and "cloud-point" determinations of the above authors as well as from preliminary trials both *in-vitro* and *in-vivo*, albumin solutions stabilized with two of the above anions were chosen as suitable for further study prior to recommendation for clinical trial. Although sodium butyrate was found to be very satisfactory as a stabilizer and to be free of toxic effects, its use was rejected because of the offensive odor of small amounts of free butyric acid present in the preparations. Sodium phenylbutyrate, while not affecting erythrocytes seriously *in vitro*, produced spastic changes in the hind limbs of mice when injected intravenously and was consequently discarded. Sodium caproate was not investigated for signs of toxicity. Sodium caprylate in the concentrations studied (0.15M to 0.3M) caused *in vitro* and *in vivo* hemolysis. The effects of human serum albumin stabilized with sodium phenylacetate and mandelate upon human red cells *in vitro*, and the consequences of the intravenous injection of these salts, with and without albumin, into mice and rabbits is submitted in the present study.

**METHODS AND RESULTS.** *Sodium Phenylacetate.* *In vitro hemolysis experiments.* One cubic centimeter solutions of standard 25 per cent human serum albumin were prepared as described by Ballou et al. (1944a) and stabilized with various concentrations of phenylacetate or phenylacetate plus sodium chloride. The pH of these preparations ranged from 6.8 to 7.2. In all samples a small amount of free acid was present in addition to the sodium salt. One cubic centimeter samples were tested for hemolytic action upon human erythrocytes at room temperature. To each sample system approximately 0.02 cc. of fresh whole human blood or twice washed human erythrocytes was added. Micro-

scopic examination of the cells, begun immediately after mixing and continued for several minutes thereafter, gave a comparison of the amount of red cell distortion produced in a sample of stabilized albumin with the appearance in a control solution. The tubes were examined for hemolysis during the first 5 minutes after setting up the system and again in 18 to 24 hours. The degree of hemolysis was estimated and recorded on an arbitrary scale ranging from zero to four plus for complete lysis of the cells. Although no hemolysis was found in any sample containing phenylacetate or mandelate, some degree of distortion of the erythrocytes occurred in all systems and is indicated in the tabulation below. Control samples of 0.15M sodium chloride alone and 0.3M sodium chloride with 25 per cent albumin were utilized. Samples of 0.15M phenylacetate with albumin produced less red cell distortion than similar samples minus the albumin, although the reverse effect was observed in the 0.3M phenylacetate systems. One sample containing 0.05M phenylacetate, 0.25M sodium chloride and 25 per cent albumin had received a preliminary heat treatment at 65°C. for 1 hour in connection with experiments (Luck, 1944) on methods of heat sterilization and removal of readily flocculable material from solutions of albumin before final filtration and packaging. This sample behaved in an identical manner in these experiments with a similar preparation which had received no heat treatment. Table 1 presents the results of the experiments with phenylacetate-albumin preparations.

*In vivo experiments.* Intravenous injections of phenylacetate-albumin preparations were made into the tail veins of unanesthetized mice in a standard dose 3 cc./kgm. body weight (comparable to the therapeutic dose in humans) or in multiples of that dosage. The general appearance and behavior of the animals as well as the rate, depth and regularity of respiration was noted for a period of five or ten minutes after each injection. No evident deleterious effects were observed in eleven mice following the injection of preparations ranging in concentration from 0.05M to 0.3M phenylacetate in 25 per cent albumin. An initial dose of 3 cc./kgm. of a 0.3M phenylacetate-stabilized albumin solution followed after fifteen minutes by a 6 cc./kgm. dose was without effect.

Two experiments were conducted with rabbits lightly anesthetized with nembutal. Arterial blood pressure from the common carotid artery, pulse rate, respiratory rate and rectal temperature were recorded. In one rabbit a solution of 0.05M phenylacetate and 0.25M NaCl in 25 per cent albumin was introduced into the external jugular vein in a dose of 3 cc./kgm. at the rate of 5 cc./min. Although the rectal temperature fell gradually during the entire experiment from 39.8° to 38.7° there was no effect on pulse rate, blood pressure or rate or depth of respiration. A second dose one half hour later similarly was without effect. A second rabbit received two comparable doses of a similar albumin preparation which had been heated at 65° for one hour. There was no change in blood pressure, heart rate, respiration or rectal temperature following the injections in this animal.

An attempt was made to find a minimum lethal dose of phenylacetate-stabilized albumin by the injection into mice of large doses of such albumin solu-

tions containing a high concentration of sodium phenylacetate. A 25 per cent albumin solution with 1.2M sodium phenylacetate was prepared. When injected into mice in a single dose of 3 cc./kgm. this preparation caused no apparent toxic effect. However, five mice which each received one dose of 6cc./kgm. exhibited transient listlessness and respiratory irregularity which disappeared within five minutes. A second dose of 6 cc./kgm. given to two of these animals

TABLE 1

*In vitro hemolysis tests. Standard human serum albumin stabilized with sodium phenylacetate*

PREPARATION	SAMPLES TESTED	WHOLE HUMAN BLOOD		WASHED HUMAN ERYTHROCYTES	
		Hemolysis	Distortion	Hemolysis	Distortion
0.15M NaCl 25% albumin	5	0	0	0	0
0.3M NaCl 25% albumin	3	0	+	0	+
0.15M phenylacetate	2	0	++++	0	++++
0.15M phenylacetate 25% albumin	3	0	++	0	++
0.15M phenylacetate 0.15M NaCl	2	0	+++	0	+++
0.15M phenylacetate 0.15M NaCl 25% albumin	2	0	++	0	++
0.3M phenylacetate	2	0	+	0	+
0.3M phenylacetate 25% albumin	3	0	++	0	++
0.05M phenylacetate 0.25M NaCl 25% albumin	1 (unheated)	0	not done	0	+
0.05M phenylacetate 0.25M NaCl 25% albumin	1 (heated 1 hr. @ 65°.)	0	not done	0	+

resulted in more evident toxicity immediately following the injection and caused death within three hours. Single doses of 3 cc./kgm. of a 1M phenylacetate-albumin system in twelve mice, 6 cc./kgm. in three mice and 9 cc./kgm. in four mice produced no apparent adverse effects. A trial of 2M phenylacetate in distilled water was made. Single 3 cc./kgm. doses into seven mice resulted in the rapid death of two of the animals and pronounced respiratory slowing and

irregularity and great listlessness in the remaining animals, although the latter recovered their normal appearance and behavior after ten minutes.

*Sodium Mandelate. In vitro hemolysis experiments.* These experiments were conducted as for the phenylacetate systems and the results are presented in table 2. In concentrations within the range suggested for clinical use from nephelometer and "cloud-point" studies (0.05M and 0.08M sodium mandelate) no hemolysis occurred and with 0.08M mandelate red cell distortion was absent.

*In vivo experiments.* Intravenous injections of the two preparations listed in table 2 produced no observable effect in doses of 3 cc./kgm. given to groups of ten mice. The 0.08M mandelate system in a dose of 9 cc./kgm. likewise caused no effect in nine mice, although transient respiratory slowing appeared in one other animal and persisted for approximately thirty seconds.

The introduction of two doses (3 cc./kgm.) of 0.08M mandelate-albumin solution one half hour apart resulted in no significant alterations of blood pressure, heart rate, temperature or respirations as recorded in two rabbits.

TABLE 2

*In vitro hemolysis tests. Standard human serum albumin stabilized with sodium mandelate*

PREPARATION	SAMPLES TESTED	WHOLE HUMAN BLOOD		WASHED HUMAN ERYTHROCYTES	
		Hemolysis	Distortion	Hemolysis	Distortion
0.04M mandelate 0.26M NaCl 25% albumin	2	0	+	0	+
0.08M mandelate 0.22M NaCl 25% albumin	2	0	0	0	0

In an attempt to determine the minimum lethal dose of sodium mandelate for mice, a 1.2M mandelate concentration in 25 per cent albumin was utilized. Doses of 3 cc./kgm. produced no effect in five mice. When the dosage was increased to 6 cc./kgm., transient respiratory slowing with rapid recovery ( $\frac{1}{2}$ –1 min.) was the only observable effect in five animals. Nine mice which received a single dose of 9 cc./kgm. all showed respiratory difficulties varying from transient slowing to complete stoppage for several seconds followed by a period of dyspnea lasting for about five minutes. Three mice received 12 cc./kgm. doses intravenously (an amount equivalent to about one-third of the animal's blood volume) and all exhibited symptoms similar to those of the previous group. The animals recovered and appeared normal twenty-four hours later.

#### CONCLUSIONS

1. Preparations of standard human serum albumin to which concentrations of phenylacetate ranging from 0.05M to 0.3M had been added caused varying degrees of distortion in the shape of human red cells *in vitro*, but no hemolysis occurred within twenty four hours at room temperature.

2. Albumin preparations stabilized with 0.04M to 0.08M mandelate caused no hemolysis, and, in the case of the latter concentration, no detectable red cell distortion.

3. No deleterious effect was observed after the intravenous injection of 3 cc./kgm. of the phenylacetate or mandelate-stabilized albumin preparations into mice.

4. Intravenous injections of 3 cc./kgm. of a 0.05M phenylacetate-stabilized albumin solution into two anesthetized rabbits caused no change in pulse rate, blood pressure and rate or depth of respiration.

5. Similar injections of 0.08M mandelate-stabilized albumin in two rabbits were without effect on pulse, blood pressure or respiration.

6. Lethal concentrations for mice of either phenylacetate- or mandelate-albumin solutions could not be prepared for injection in any volume consistent with the animal's blood volume.

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# EFFECTS OF CHYMOTRYPSIN ON INSULIN AND BLOOD GLUCOSE

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It has been shown that insulin is digested *in vitro* by many proteolytic enzymes and that its hypoglycemic action is destroyed by such enzymes (1, 2). Pepsin, trypsin, papain and cathepsin inactivate insulin *in vitro*, while aminopolypeptidases, protaminase, carboxypeptidases and dipeptidases do not, apparently because insulin does not constitute an adequate substrate for their action (3, 4). There are few reports on the action of proteolytic enzymes on insulin *in vivo*, although the suggestion has been made frequently that proteolytic enzymes may be responsible for the destruction of insulin in the body tissues and fluids, both in normal and pathological conditions (1, 2). Furthermore it is known that insulin is destroyed by the enzymes of the digestive tract, which explains the fact that it is inactive when given *per os*. Buckley (5) observed transient elevation of the blood sugar after subcutaneous injection of a single dose of a trypsin preparation into rabbits and suggested that this effect could be explained by a destruction of insulin by trypsin *in vivo*. However, trypsin, injected into animals, has many other effects, among which are the production of necrosis of the skin and the production of a special type of shock (6). It is conceivable that some of these effects would explain the hyperglycemic action of trypsin, without there being a direct action of trypsin on insulin *in vivo*.

The present communication reports the results of further investigation of the effect of proteolytic enzymes on insulin *in vitro* and on the blood glucose of experimental animals. The proteolytic enzyme used in this work was chymotrypsin.

**MATERIAL AND METHODS.** Crystalline chymotrypsin prepared according to the method of Kunitz and Northrop (7) was used in all experiments. It has the following advantages: chymotrypsin is available in very pure form, is optimally active at the pH of the blood (7), is much less toxic than trypsin, and consequently larger doses, having more proteolytic activity, can be injected without killing the experimental animals (8), and it is active *in vivo* since it causes digestion of the blood fibrinogen when it is injected intravenously (8).

The crystalline chymotrypsin<sup>2</sup> which contained about 50 per cent MgSO<sub>4</sub> by weight was dissolved in 5 to 20 ml. of physiological saline solution, and injected intravenously into 9 dogs and 22 rabbits. The dogs were anesthetized by 0.4 ml. per kgm. of a 10 per cent aqueous solution of sodium pentobarbital given by vein. The rabbits were not anesthetized. All animals were fasted for 14 to 24 hours before the experiment was begun.

<sup>1</sup> Died January 3, 1945.

<sup>2</sup> Obtained from the Plant Research Laboratory, Bloomfield, N. J.



Blood glucose determinations were made on the dog blood by the Somogyi-Benedict method (9) and on the rabbit blood by the micro-method of Folin-Malmros as adapted for the Evelyn photoelectric colorimeter (10). pH determinations were made with a glass electrode and using a Beckman pH meter.

RESULTS. 1. *The inactivation of insulin by chymotrypsin in vitro.* Fifty units of insulin were incubated at 37°C. and at pH 7.1 with 10 mgm. of chymotrypsin in a total volume of 25 ml. Five milliliters of the mixture, representing 10 units of insulin, were injected intravenously into rabbits after varying periods of time. Blood glucose determinations were made, and the rabbits were observed for convulsions. The results (table 1) show that the insulin was completely inactivated by chymotrypsin within 30 minutes.

TABLE 1  
*Effect of chymotrypsin on insulin in vitro*

RABBIT NO.	WEIGHT	MIXTURE A* QUANTITY INJECTED	MIXTURE B† QUANTITY INJECTED	BLOOD GLUCOSE				CONVULSION‡
				0	1 hr.	3½ hr.	4 hr.	
	kgm.	ml.	ml.	mgm./100 ml.				
1	1.8	5		100	120	100		0
2	1.9	5		94	90	88		0
3	1.8	5		98	103	102		0
4	1.7	5		75	103	110		0
5	1.9	5		87		116		0
6	2.6	5		123			100	0
7	2.8	5		67			85	0
8	2.1	5		60			105	0
9	1.9		4.5	84				+ (at 80 min.)
10	1.9		4.5	93				+ (at 45 min.)

\* A: 50 units insulin + 10 mgm. chymotrypsin + 3 ml. phosphate buffer + H<sub>2</sub>O to make 25 ml. incubated at 37°C. and pH 7.1.

† B: 15 units insulin + 1 ml. phosphate buffer + H<sub>2</sub>O to make 10 ml. incubated at 37°C. and pH 7.1.

‡ Every animal was observed for convulsions for 5 hours after the injection.

2. *Effect of chymotrypsin on the hypoglycemic effect of insulin in rabbits.* When 10 units of insulin and from 15 to 39 mgm. per kgm. of chymotrypsin were injected simultaneously into opposite ear veins in 6 rabbits, the usual hypoglycemic action of insulin was observed. In 5 of the rabbits the insulin caused hypoglycemic convulsions, and in the other rabbit the blood sugar was lowered to 29 mgm. per 100 ml. two hours after the injection of the chymotrypsin.

3. *Effect of intravenous injection of chymotrypsin on the blood glucose.* In 5 fasting and unanesthetized rabbits the changes in blood glucose following the injection of from 25 to 27 mgm. per kgm. of chymotrypsin were not significant. The only possible exception occurred in one rabbit where a progressive rise in the blood glucose level to a maximum of 135 mgm. per 100 ml. was observed when 25 mgm. per kgm. of chymotrypsin were injected intravenously and the injection was repeated 30 minutes later. In one of two control rabbits receiving saline

intravenously instead of chymotrypsin, the blood glucose level went higher than in the other animals (142 mgm. per 100 ml.).

In 12 experiments chymotrypsin was injected intravenously into fasting un-anesthetized dogs. The blood glucose level did not change significantly after the injection in 7 experiments. In 5 experiments there was a slight rise in blood glucose, which never exceeded a level of 145 mgm. per 100 ml. of blood (table 2, expts. 1, 3, 4, 5, 7). The rise in blood glucose was observed to appear 60 to 240 minutes following the injection of chymotrypsin.

In 2 dogs 50 mgm. per kgm. of  $MgSO_4$  were injected, amounting to more than twice the maximum quantity of  $MgSO_4$  that was given when the chymotrypsin

TABLE 2  
*Effect of chymotrypsin on the blood sugar of dogs*

EXP. NO.	DOG NO.	WEIGHT	CHYMOTRYPSIN	BLOOD SUGAR														
				Before injection				Minutes after injection										
								60	30	15	0	15	30	45	60	90	120	150
		kgm.	mgm./kgm.	mgm./100 ml.														
1	1	16	20		95	102	98	110	99	108	114		138		129			
2	2	9	25	94	91		93		86		87	71	78	81			91	
3	3	7.7	25	94	70		83		74		112	112	112		124		124	
4	4	8.8	25	63	86		66		70		66	88.5	120		109		107 107	
5	1	16	25	125	117					116		129.5	135		145		99 123.5	
6	2	9.1	25	87	93					82.4		100		103	100		88 100	
7	3	8.5	26	65			65.5				54			81.5				
8	7	10.5	26		86		92				110		110	121			102	
9	8	7.5	26		93		84				66.5		76.5	77			105 106	
10	9	15	15*	100					102.6		102.6		111.2	111.2	108.2			
11	8	8.1	28				83.6				95.2		87	77	77		87	
12	12	7.8	26		92.7				92.7		79.7	87.9	90	88	82			

\* The chymotrypsin was injected intramuscularly into this animal.

Intravenous injections lasted 2 to 3 minutes, except in dog 4 where the injection lasted 1 hour.

preparation containing  $MgSO_4$  was injected. No effect on the blood glucose was observed following this quantity of  $MgSO_4$ .

The possibility was investigated that chymotrypsin, by proteolysis of plasma proteins, might liberate non-sugar reducing substances that would be calculated as glucose in the sugar determination. This could have explained the slight rise in blood sugar level observed in 5 of the dogs. Ten cubic centimeters of oxalated blood, mixed with sodium fluoride, were incubated with 20 mgm. of chymotrypsin, and blood glucose determinations were made at intervals by the Somogyi-Benedict method. No change in the glucose level of such blood was demonstrable over a period of 4 hours.

DISCUSSION. The data reported here show that chymotrypsin inactivated insulin *in vitro*, presumably by proteolyzing it. According to Fruton and Berg-

man (11) chymotrypsin acts as an aminopeptidase and as a carbonylproteinase. Since it has been found that aminopeptidases have no inactivating effect on insulin (3, 4), it would appear that the carbonylproteinase action of chymotrypsin is responsible for the inactivation of insulin.

The action of chymotrypsin on insulin *in vitro* suggested the possibility that chymotrypsin could have a hyperglycemic effect when injected intravenously. However, no significant or constant effect on the blood sugar level was observed after intravenous injections of from 20 to 28 mgm. per kgm. of chymotrypsin into dogs or from 25 to 27 mgm. per kgm. into rabbits. Similarly, such injections did not abolish the hypoglycemic effect of 10 units of insulin administered to rabbits at the same time. Larger doses of chymotrypsin were not used because they often are lethal. The absence of significant effect of chymotrypsin *in vivo* on the blood glucose level cannot be explained by an absence of proteolytic activity of chymotrypsin *in vivo*, since it has been demonstrated that proteolytic activity is present in the circulating blood for as long as 40 minutes after intravenous injection of quantities of chymotrypsin similar to those used in this work (8).

The experimental evidence presented here does not rule out the possibility that, by other methods of administration, one could demonstrate a proteolytic action of chymotrypsin on insulin *in vivo*. Too little is known about the secretion and fate of insulin in the blood to permit a more positive conclusion. However, the data indicate that a single injection of a maximal dose of a proteolytic enzyme that is able to digest insulin *in vitro* and that persists in the circulating blood for at least 40 minutes has no effect on the blood sugar level and does not protect against the hypoglycemic action of 10 units of insulin given at the same time.

Pathologic examinations of the tissues of the dogs that received intravenous injections of chymotrypsin showed central necrosis of the liver and focal necrosis in the adrenal cortex in some of the animals (8). These findings might have significance in relation to the slight elevation of the blood sugar level sometimes observed after injection of chymotrypsin.

#### SUMMARY

1. Chymotrypsin inactivates insulin *in vitro*.
2. Chymotrypsin given intravenously in doses of from 20 to 37 mgm. per kgm. had no significant effect on the blood glucose level of rabbits and dogs.
3. Simultaneous injections of 25 mgm. per kgm. of chymotrypsin and 10 units of regular insulin into rabbits were followed by the usual hypoglycemic action of insulin alone.

The technical assistance of Henry Krakauer is gratefully acknowledged.

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# THE NATURE OF PUPILLARY DILATATION IN ANOXIA<sup>1</sup>

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In a preceding paper Ury and Gellhorn (1939) showed that the pupillary dilatation in the cat following painful stimuli is due to a diminution of the tone of the third nerve center. This was proven by the fact that when the third nerve was cut intracranially, and the pupil was constricted by physostigmine, no dilatation was observed on this eye after application of painful stimuli although the pupil reacted to minimal stimulation of the cervical sympathetic. These results were confirmed by Seybold and Moore (1940) as well as Hodes (1940). The latter author performed, in addition, some experiments on the effects of anoxia on the pupil which led him to conclude that pupillary changes were observed only when the animals were moribund. Moreover, these changes were said to be caused by direct effects of anoxia on the iris and not by neural factors. In view of the fact that Ury and Gellhorn (1939) had seen clear evidence of pupillary dilatation in moderate degrees of anoxia in the rabbit and that similar changes had been observed in the cat by the senior author a reinvestigation of this problem was deemed of interest.

The experiments were performed on cats anesthetized with 0.45 cc. dialurethane<sup>2</sup> per kgm. intraperitoneally. The pupillary changes occurring in a low pressure chamber (anoxia) as well as after clamping of the trachea (asphyxia),<sup>3</sup> were studied on normal pupils, after sectioning of the cervical sympathetic on one side, after intracranial sectioning of the third nerve and additional cervical sympathectomy on the same side, and after adrenalectomy. Some animals were observed 4 to 6 weeks, after intracranial section of the third nerve, whereas in other animals the effect of anoxia was studied on the acutely denervated iris about one hour after sectioning of the third nerve and unilateral cervical sympathectomy.

**RESULTS.** At the outset it may be stated that anoxia and asphyxia induce pupillary dilatation of varying degrees including maximal dilatation. It is a reversible phenomenon in the cat and may be studied many times in the same animal. It is certainly not a "terminal" phenomenon. However, in order to perform experiments of this kind artificial respiration must be instituted. In general, it was found that a distinct pupillary dilatation was observed at a simulated altitude practically identical with that at which respiratory failure occurred. In some experiments in which no artificial respiration was administered, and in which the pupils dilated maximally, or nearly maximally, it was possible

<sup>1</sup> Aided by a grant from the Josiah Macy Jr. Foundation.

<sup>2</sup> Kindly supplied by Ciba Pharmaceutical Products, Inc., Summit, N. J.

<sup>3</sup> As to the distinction between anoxia and asphyxia cf. Gellhorn and Lambert, 1939.

to restore spontaneous respiration by rapid decompression; in other instances artificial respiration had to be applied. In either group the pupillary dilatation was found to be reversible provided that respiratory failure was prevented. On the basis of these observations our subsequent experiments were performed under conditions of artificial respiration.

I. *The rôle of the sympathetic in the dilatation of the pupil in anoxia.* Systematic observations on the effect of low barometric pressure on the normal and sympathectomized pupil do not give any evidence of sympathetic excitation of the dilator pupillae under these conditions. This statement is founded on the fact that the "threshold pressure"<sup>4</sup> is practically identical for the normal and sympathectomized pupil. Thus it was found that at a rapid rate of ascent to 15,000 and 20,000 feet, which level was reached in 100 and 140 seconds respectively, the normal as well as the sympathectomized pupil remained unchanged. If the barometric pressure was lowered to 25,000 feet both pupils dilated slightly in most instances, and ordinarily the normal more than the sympathectomized pupil (table 1a). There is a high degree of parallelism between the curves representing the dilatation of the normal and sympathectomized pupil. This is illustrated likewise in table 1b, in which the cat was exposed to a barometric pressure of 27,000 feet for six minutes. Within the first minute at this altitude both pupils dilated. Thereafter the pupillary dilatation decreased until, toward the end of the period of reduced pressure, a second wave of dilatation occurred. This parallel behavior of both eyes was seen in all experiments although the difference in the degree of pupillary dilatation between the normal and the sympathectomized eyes varied considerably. In one experiment the normal and sympathectomized pupil dilated to exactly the same degree, and at the same rate, from 2 to 12 mm. while exposed to a lowered barometric pressure and this dilatation was completely reversible. In the majority of experiments, however, the normal pupil dilated to a greater extent than the sympathectomized. Table 2a shows almost no difference in rate and degree of dilatation between the normal and sympathectomized pupil during the ascent to 29,000 feet, but during descent the normal pupil remains slightly more dilated than the sympathectomized. Table 2b shows a distinct difference in the degree of dilatation between the two pupils, but in spite of it there is a high degree of parallelism between the curves with respect to the onset of the dilatation during the ascent, the time of reversal and the gradual decrease in pupillary diameter. Moreover, it was found in many experiments that elimination of sympathetic innervation of the iris does not prevent the occurrence of maximal dilatation in anoxia, as table 2b illustrates. These phenomena suggest that the cervical sympathetic influences the pupil only on account of its tone. If the latter is nil the rate and degree of pupillary dilatation is the same in the normal and sympathectomized pupils; otherwise considerable differences between the two pupils may exist. It is worthy of note that pupillary difference based on the absence of sympathetic innervation of

<sup>4</sup> Threshold pressure = highest barometric pressure which at a given rate of ascent causes a perceptible degree of pupillary dilatation.

one pupil may appear during the ascent in the low pressure chamber although both pupils are maximally constricted at normal barometric pressure. Apparently, the sympathetic tone which may be unable to oppose the maximal constrictor tone of the third nerve center at sea level when the parasympathetic tone is greatest, shows its effect on the pupil with increasing degree as the third nerve tone is diminished with decreasing barometric pressure.

TABLE 1

*Influence of lowered barometric pressure on the diameter of the pupil*

TIME IN MIN.	ALTITUDE IN FEET $\times 10^3$	NORMAL PUPIL	SYMPATHECTOMIZED PUPIL
A			
		mm. diameter	
0	0	0.5	0
1.5	15	0.5	0
2.0	20	0.5	0
3.0	25	3.0	1.5
3.5	30	10.0	7.0
4.0	25	6.0	4.0
6.0	20	4.0	2.0
6.6	15	2.0	0
8.6	0	0	0
B			
0	0	0.5	0
2.5	20	1.0	0
3.2	25	2.0	0.5
4.0	27	6.5	3.0
5.0	27	4.0	2.0
6.0	27	4.0	2.0
7.0	27	4.5	3.0
8.0	27	4.5	3.0
9.0	27	6.0	4.0
9.5	27	11.0	9.0
10.0	20	12.0	11.0
12.0	10	7.0	4.0
13.3	0	3.0	1.0
15.0	0	1.0	0

TABLE 2

*Influence of lowered barometric pressure on the diameter of the pupil*

TIME IN MIN.	ALTITUDE IN FEET $\times 10^3$	NORMAL PUPIL	SYMPATHECTOMIZED PUPIL
A			
		mm. diameter	
0	Sea level	0	0
2.3	20	0	0
4	26	1.5	1.5
7	29	3.0	3.0
7.5	29	7.0	7.0
8	29	11.0	11.0
8.5	15	12.0	12.0
9	8	8.0	5.0
10	Sea level	4.5	3.0
11	Sea level	4.0	2.0
13.2	Sea level	1.5	1.0
B			
0	Sea level	0	0
3	25	0	0
5	27	2.0	0.5
7	29	2.0	1.0
9	31	2.5	1.0
10	31	4.5	3.0
12.5	32	6.0	4.5
13	32	8.0	5.8
13.5	10	12.0	12.0
13.8	Sea level	9.0	8.0
14	Sea level	5.0	5.0
14.8	Sea level	0.5	0.5

Another point already studied by Hodes may be briefly mentioned. There is no evidence that adrenalin secretion has a distinct effect on the pupillary dilatation observed in the low pressure chamber. Maximal reversible pupillary dilatation was observed in the normal and sympathectomized pupils at low barometric pressures in normal as well as in acutely adrenalectomized animals. By comparing the effect of low barometric pressures on the pupil before and after ad-

renalectomy no greater variations were found than were seen in successive experiments performed on the same animal without any surgical interference.

II. *The pupillary dilatation in the completely denervated eye.* In four cats the third nerve was cut intracranially and the pupil constricted by local application of physostigmine (cf. Ury and Gellhorn). Moreover, the cervical sympathetic was cut at the same side immediately prior to the experiment. Therefore, one pupil was completely denervated and its reaction was compared with the normal pupil of the other side during the lowering of barometric pressure. Figure 1 illustrates a typical experiment in a cat in which the sectioning of the third nerve had been performed one month earlier. Two phenomena were quite obvious and occurred consistently in each of the experiments: first, the completely denervated pupil showed a slightly increasing dilatation which in rate and degree was quite different from that seen in the normal or sympathectomized eye; secondly, in no

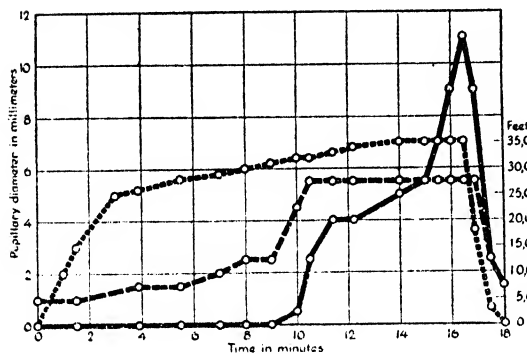


Fig. 1

Fig. 1. Effect of lowered barometric pressure on the pupillary diameter of the cat. — normal pupil; — — — completely denervated pupil; --- altitude.

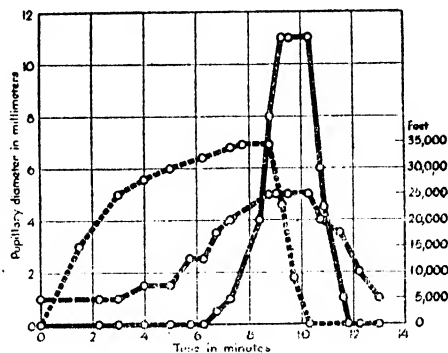


Fig. 2

Fig. 2. Effect of lowered barometric pressure on the pupillary diameter of the adrenalectomized cat. — sympathectomized pupil; — — — completely denervated pupil; --- altitude.

instance was a maximal reversible dilatation of the denervated eye obtained in anoxia. Figure 1 shows that a simulated altitude of 35,000 feet led to a pupillary dilatation of 11 mm. on the normal, but only of 5.5 mm. on the denervated pupil. In other experiments recorded in table 3 similar differences were observed. As this table shows, this result holds for normal as well as acutely adrenalectomized animals.

In another cat in which the right pupil was completely denervated a series of experiments was performed in the low pressure chamber. In successive experiments of this series the effect of anoxia was studied on the left pupil: first, when it was normal, then after its sympathetic supply had been eliminated, and finally, after the adrenals had been removed. In agreement with earlier observations no effect of these procedures was seen on the reactivity of the parasympathetically intact pupil. Figure 2 represents one experiment of this series, which is of particular interest since it gives evidence of the rôle of the third nerve in pupillary



dilatation during anoxia. In the experiment represented in this figure both eyes were sympathectomized, and the hormonal compound of possible physiological significance (adrenalin) was eliminated by adrenalectomy. Since the third nerve is intact in the left eye whereas it has been sectioned previously in the right, the difference in the pupillary diameter between the two eyes is a quantitative measure of the contribution of the third nerve to the anoxic dilatation of the pupil. The record shows clearly that the major factor in anoxic pupillary dilatation is a diminution of the parasympathetic tone.

These experiments were confirmed by studies on the effect of asphyxia induced by clamping of the trachea (fig. 3). The results on four cats with either chronically or acutely sectioned third nerves on one side showed again that the completely denervated pupil reacted to asphyxia with a relatively slow dilatation of moderate degree, whereas the normal or sympathectomized pupil dilated maxi-

TABLE 3

CAT NO.	ALTITUDE IN FEET $\times 10^3$	NORMAL PUPIL	DENERVATED PUPIL	
			<i>mm. diameter</i>	
I	29	10	4	
	32	10	5	
	35	12	6	
	35	11	5.5	After adrenalectomy
	36	11	6	After adrenalectomy
	36	12	5.5	After adrenalectomy
II	34	11	3.5	
	35	11	5	
	35.5	11	5	After adrenalectomy
	34.5	11	5	After adrenalectomy
III	31	12	5	
	33	10	3	

mally and at a faster rate. There was, however, an interesting difference in the degree of dilatation of the denervated eye of various cats which seemed to depend on the time elapsed since the third nerve had been cut. In the acutely denervated eye the pupil attained a diameter of 2.5 to 4 mm. while the normal or sympathectomized pupil dilated maximally to 11 to 13 mm. Under the same conditions the chronically denervated pupil dilated to 4.5 to 5.5 mm.

These differences persisted when asphyxia continued until death occurred. Figure 4 shows the course of pupillary dilatation during fatal asphyxia for three animals. The curves obtained for the eyes whose parasympathetic innervation was intact showed the well known rapid dilatation of the pupil occurring in asphyxia (left pupil). The rate and degree of dilatation seen in the left pupils is apparently independent of the sympathetic innervation of the eye. It likewise indicates that the secretion of adrenalin plays no measurable rôle. The right (parasympathectomized) pupil behaved quite differently. In only one out of

four cases did the pupil become maximal in fatal asphyxia. In the other cases the final pupillary dilatation was 8.5, 6<sup>5</sup> and 3.5 mm. It is interesting to note there is a definite relation between the maximal pupillary size observed at death in parasympathectomized pupils and the time which had elapsed since the third nerve had been cut. The parasympathectomized pupils which dilated to 12 and 8.5 mm. had been denervated 68 and 40 days respectively prior to the experiment, whereas the lesser degrees of dilatation at death (3.5 and 6 mm.) were observed on cats in which the third nerve had been cut only a few hours before the experiment was performed. The graphs indicating the pupillary dilatation of the parasympathetically innervated eye showed a rapid and uniform course which leads in a few minutes to a maximal and irreversible dilatation. On the

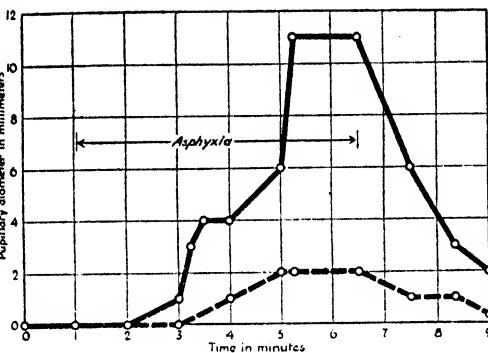


Fig. 3

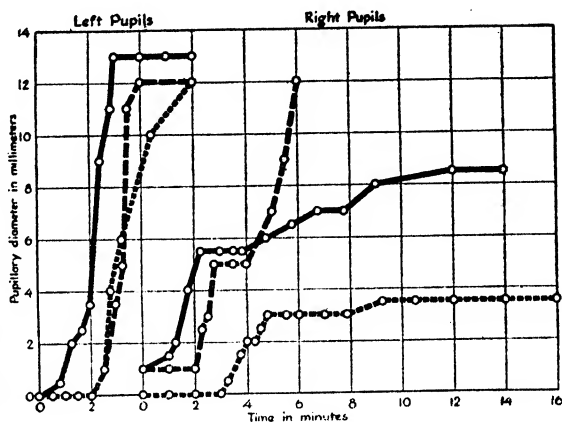


Fig. 4

Fig. 3. Effect of asphyxia on the normal ——— and the completely denervated — — — pupil. The 3rd nerve had been sectioned just prior to the experiment.

Fig. 4. The effect of irreversible asphyxia on the pupil.

Left pupil ——— normal; right pupil ——— completely denervated.

Section of 3rd nerve 40 days prior to the experiment; adrenalectomized cat.

Left pupil — — — sympathectomized; right pupil — — — completely denervated.

Section of the 3rd nerve 68 days prior to experiment; adrenalectomized cat.

Left pupil --- normal; right pupil --- completely denervated a few hours before experiment; normal cat.

other hand, the changes recorded on the denervated pupils showed three definitely separate phases. The first leading in 3 or 4 minutes to a dilatation of 3 to 5 mm. corresponds to the reversible dilatation seen in asphyxia and anoxia in the denervated eye. The second phase consisted of a plateau in which this degree of dilatation was maintained for several minutes whereas the third phase caused a gradual increase to the final degree, the magnitude of which seemed to depend on the time which had elapsed since the sectioning of the third nerve had been performed.

DISCUSSION. The experiments reported in this paper show that the pupillary

<sup>5</sup> The record of this experiment is omitted from figure 4.

dilatation observed in anoxia and asphyxia is largely due to a diminution of the tone of the third nerve. It was also shown that even the irreversible dilatation of the pupil occurring in fatal asphyxia is based to a large extent on the same neural mechanism. In this respect our results are contrary to Hodes, whereas with regard to the rôle played by the sympathetic and the adrenal medulla we agree with this author. In the light of these experiments the statement seems to be justified that the neural mechanism of pupillary dilatation after administration of painful stimuli is similar to that seen in anoxia. In both instances neither the sympathetic innervation nor the secretion of adrenalin plays a rôle. However, there are two important differences; first, in the pain experiments described earlier by Ury and Gellhorn the "denervated" pupil remains unchanged whereas moderate degrees of dilatation are attained in anoxia and asphyxia. Secondly, pain results in a temporary inhibition of the tone of the third nerve through reflex inhibition whereas anoxia and asphyxia weaken the tone of the third nerve center directly so that a given degree of illumination no longer produces any constriction of the pupil.

The fact that the "denervated" pupil dilates in anoxia induced Hodes to conclude that the pupillary dilatation in anoxia is "not of neural origin." The present experiments indicate that this is only partially true. At least one half of the dilatation seen in anoxia on the denervated side is due to a reversible loss of tone of the third nerve. However, in addition, a non-neural component is active on the denervated side which causes a slow, moderate dilatation to occur in anoxia. Since this phenomenon is retained even after adrenalectomy, neither a neuronal nor a known hormonal factor seems to be involved. This suggests that local effects on the iris are the cause of the dilatation seen in the denervated anoxic pupil. Since it is well known that in anoxia and asphyxia acid metabolites are formed at a greatly increased rate, and since such metabolites may cause reversible as well as irreversible contractures in striated and smooth muscles, they may well be responsible for the non-neural part of dilatation seen in anoxia. However, this interpretation does not account for the as yet unexplained differences in pupillary dilatation which appear to be related to the period of denervation of the third nerve.

The experiments described in this paper have without doubt established the fact that parasympathetic inhibition is an essential part of the pupillary dilatation seen in anoxia and asphyxia, but the writers feel some doubt as to the physiological validity of the pupillary dilatation based on non-neural factors. A close study of our records shows that in the chronic preparation the parasympathetically denervated pupil shows an earlier onset of the dilatation on the denervated as compared to the normal side, whereas in the acutely denervated pupil the non-neural dilatation seen in anoxia and asphyxia is not only quantitatively smaller but its onset is actually delayed when compared with the curve of pupillary dilatation seen in the normal eye. These observations suggest that the iris undergoes some fundamental changes as a result of the parasympathetic denervation. Consequently, the iris becomes responsive to non-neural factors (metabolites?) produced in anoxia although these same factors have either no or only insignificant effects on the normal pupil.

It was mentioned earlier that the proof for the occurrence of a maximal dilatation of the pupil in anoxia and asphyxia depended on the maintenance of artificial respiration. It seemed that the dilatation of the pupil which, as our analysis showed, was due to an inhibition of the tone of the third nerve nucleus, occurred about at the time when the respiration failed. It is assumed by most authors that cortex and midbrain are more sensitive to anoxia than are the medullary centers. However, the fact that the experiments described in this paper were performed under barbiturate anesthesia may well explain this difference.

Our experiments show clearly that the neural component of the dilatation occurring in anoxia and asphyxia consists in a diminution of the central tone of the third nucleus without any alteration in the sympathetic innervation of the pupil. Apparently the principle of reciprocal innervation<sup>6</sup> is not applicable to the pupillary changes which appear under conditions of anoxia or asphyxia, and as a result of pain. It remains to be seen whether the pupillary dilatation in deep narcosis is likewise solely the result of a diminished central tone, or if under these conditions sympathetic excitation of the dilator mechanism is involved at the same time.

#### SUMMARY

Experiments were performed in normal and adrenalectomized cats on the size of the pupil under the influence of anoxia produced by lowering of the barometric pressure, and as the result of asphyxia induced by clamping of the trachea. The pupillary size was determined on normal, sympathectomized, and completely denervated pupils. No evidence was found that either adrenalin or sympathetic excitation plays a part in the dilatation of the pupil seen in anoxia and asphyxia. The pupillary dilatation under these conditions may be maximal and completely reversible provided that artificial respiration is maintained. The physiological analysis shows that the dilatation of the pupil consists in a neural and a non-neural component. The former is due to a diminution of the tone of the third nerve center; the latter is probably associated with the formation of acid metabolites.

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<sup>6</sup> For further discussion cf. E. Gellhorn, p. 217.

# THE MEASUREMENT OF THE BLOOD PRESSURE IN RATS WITH SPECIAL REFERENCE TO THE EFFECT OF CHANGES IN TEMPERATURE

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During the course of studies of experimental hypertension in rats, marked variability of the systolic blood pressure was observed under otherwise similar conditions, when the measurements were made at different environmental temperatures and with and without barbiturate anesthesia. It became desirable therefore to appraise the extent of the influence of certain types of anesthesia and of heating the animals in view of the widespread practice of anesthetizing or heating rats prior to recording blood pressure.

Little attention has been paid to the fact that many of the reported records were secured under abnormal environmental conditions. Yet, it is generally accepted that the application of external heat alters the cardiac and respiratory rates, the basal metabolic rate, and the body temperature in laboratory animals (1-4). Doubt concerning the validity of blood pressure measurements in rats was expressed by Schroeder (5). The divergent and inconsistent results obtained by both direct and indirect methods of measurement led Schroeder to believe that "the weight of the heart is probably a more reliable index of the presence of hypertensive state than is one measurement, or two, of blood pressure under anesthesia." Having observed a pressor effect following the application of heat, we have made a study of the influence of changes in body temperature upon the blood pressure. Observations of the rôle of barbiturate anesthesia have also been made because of the depressing effect of anesthesia on body temperature.

**METHODS.** All experiments were done between November and June. The rats were of a Wistar strain. They weighed 125 to 320 grams and were 2½ to 10 months old. Approximately half of the rats were males. The diet was Purina Checkers. To make some of the animals hypertensive, capsules of cellophane were placed around one or both kidneys (6).

Two methods were used for the measurement of blood pressure. One is the indirect method of Byrom and Wilson (7) which measures the pressure in an inflatable cuff applied to the base of the tail which just permits blood to flow into

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<sup>4</sup> On leave of absence for military service.

that part. The length of the cuff used in our experiments was 4 cm. The entrance of blood is detected by noticing an increase of the volume of the tail with a plethysmograph connected with an oncometer tube 1 mm. in diameter. After the tail is sealed into a rigid chamber, the plethysmograph is filled with water of the desired temperature. The animal is held in a container of body size, with walls of sheet metal or wire mesh. Since anesthesia is usually not employed in the use of this method, the animals are trained daily for at least a week before the measurements of blood pressure are considered accurate.

The other method involves the use of the optical manometer of Hamilton, Brewer and Brotman (8) and the insertion of a hollow needle into a femoral artery. It was necessary to induce and maintain general anesthesia whenever this method was used. For this purpose, 5 mgm. of sodium pentobarbital (nembutal) per 100 grams of body weight were injected intraperitoneally.

Thermocouples placed in the rectum and on the shaved skin of the abdomen were connected with a recording galvanometer for the measurement of internal and cutaneous temperatures. Heating the animals preparatory to or during the measurement of blood pressure was accomplished by exposing them to two 150 watt electric bulbs or coils of nichrome wire. To decrease the amount of radiant energy in some instances, a cloth covered tent six inches high with vents at each end to permit access of fresh air was placed over the animals. When it was desired to maintain rather than elevate body temperature, the animals were placed on an electric pad. Cooling was accomplished either by discontinuing application of external heat or by exposure to a stream of cool air from an electric fan.

**OBSERVATIONS.** The range of systolic pressure in 24 animals measured at room temperature (24–28°C.) by the plethysmographic method was from 60 to 95 mm. Hg; in three-fourths the range was 65 to 90 mm., and in a few instances no readings could be obtained. In these experiments the temperature of the water in the plethysmographic chamber surrounding the tail was 32.5° to 34°C. To study the effect of the temperature of this water upon the blood pressure, three series of 50 readings were made on each of 7 rats over a period of a week. In the first series, the water was maintained at 32.5° to 34°C.; the range of the systolic blood pressure in 33 readings was from 60 to 90 mm.Hg, in 8 trials the readings were equivocal and in 9 no readings could be obtained. In the second series, the water was maintained at 36.4° to 38.6°C.; the range of blood pressure in 46 readings was from 65 to 95 mm.Hg, the mode 70 to 85; in 4 trials the readings were equivocal. In the third series the water was maintained at 32.5° to 34°C. and the animals were exposed to an environment of 40°C. for three minutes before the readings were made. A rise in body temperature of 0.2° to 0.5°C. occurred; the range in blood pressure was from 75 to 95 mm.Hg in 47 readings; in 3 trials the readings were equivocal.

The effect of elevation of body temperature on the level of blood pressure was studied in twelve unanesthetized and 4 anesthetized normal rats by use of the plethysmographic method. Exposure to enough radiant energy to cause a rapid rise in both cutaneous and rectal temperatures was associated with a

progressive elevation of blood pressure of 10 to 80 mm.Hg within a mode of 6 to 10 minutes (fig. 1). The increase of rectal temperature in the unanesthetized rats was  $0.4^{\circ}$  to  $2.0^{\circ}\text{C.}$ , for the anesthetized animals  $3.0^{\circ}$  to  $4.8^{\circ}\text{C.}$  In another group of experiments with 10 unanesthetized and 6 anesthetized rats the exposure to radiant energy was such as to require a mode of 16 to 42 minutes for comparable rises in rectal temperature. This slower elevation of body temperature was nevertheless associated with a progressive elevation of blood pressure of 23 to 106 mm.Hg, essentially the same increase as occurred with the more rapid rise in temperature. No matter how slowly the elevation in body tempera-

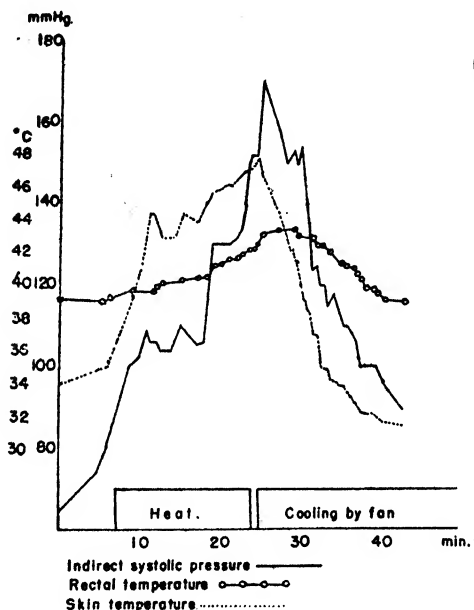


Fig. 1

Fig. 1. Increase of rectal temperature, cutaneous temperature, and blood pressure during heating, followed by decline upon rapid cooling. The rise of blood pressure and cutaneous temperature before the application of heat is attributable to the insulating effect of the holder with solid walls. (Normal rat, unanesthetized, indirect method.)

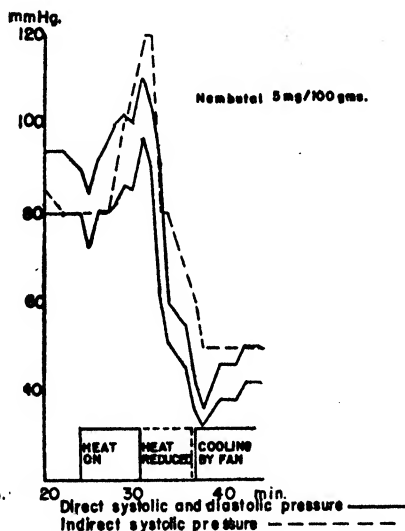


Fig. 2

Fig. 2. Simultaneous records of the blood pressure obtained by direct and indirect methods; blood pressure altered by heating and cooling a normal anesthetized rat. The changes obtained by both methods parallel each other.

ture was achieved, the greater part of the accompanying rise in blood pressure occurred during the first ten minutes after the application of heat. Results similar to those described for normal animals were obtained when hypertensive animals were studied or when the intra-arterial method for recording blood pressure was used. This was done in 9 experiments. The tracings reveal that increase of blood pressure was accompanied by a concomitant increase of cardiac rate.

A rise in body temperature sufficient to be associated with a rise in blood pressure occurred whenever an animal was held in an unheated container with walls of sheet metal prior to or during the recording of blood pressure (fig. 1).

No such rise in body temperature occurred when a container with walls of wire mesh was used.

Continuous prolonged heating of 3 rats resulted in marked elevation of rectal and cutaneous temperatures. This was accompanied by elevation of blood pressure from 175 to 226 mm.Hg and marked increase of cardiac rate, followed by sudden fall of blood pressure to levels too low to measure accurately and death of the animals within 40 to 160 minutes of continuous heating.

The effect of lowering the body temperature was observed under a variety of conditions. When the temperature of animals that had been heated was allowed to return to normal, the blood pressure concurrently returned to normal. The effect of depressing cutaneous and rectal temperatures to below normal was studied by exposing the animals to a draft of cool air. This manoeuvre was insufficient to produce the desired effect in unanesthetized animals and in them no additional fall in blood pressure occurred. In anesthetized animals, however, a fall in cutaneous and rectal temperatures to below normal occurred, accompanied by a progressive fall in blood pressure to below normal and then by a secondary rise in spite of continued depression of temperature. In one instance, the systolic pressure fell from 175 mm.Hg (induced by heat) to 104 mm.Hg in 26 minutes of cooling, followed by a rise to 160 mm.Hg during the next 16 minutes of cooling. Exposure to environmental temperature (24–28°C.) in the absence of the additional cooling effect of a stream of air from a fan depressed the temperature of 13 anesthetized normal rats and 20 anesthetized rats with perinephritis from 0.4° to 1.9°C. within 27 minutes. As measured by both the indirect and direct methods, this was accompanied by a fall in blood pressure of 10 to 25 mm.Hg. In 6 animals, followed for 2 hours, the fall in blood pressure was succeeded by a secondary rise which, except in one instance, exceeded the preanesthetic level. In the tracings made with the direct method the heart rate decreased and increased synchronously with the fall and subsequent rise of blood pressure.

Simultaneous recording of blood pressure by the indirect and direct methods was undertaken in four experiments (fig. 2). Because the intra-arterial methods require the use of anesthesia, no simultaneous records could be obtained in unanesthetized animals. The systolic readings obtained by both methods agreed within 15 mm.Hg in the normal range as well as when the blood pressure was deliberately altered by heating or cooling the animals. Detecting changes in blood pressure was more prompt with the direct method. Shuler, Kupperman and Hamilton (9) have recently reported on the comparison of the indirect and direct methods in a series of normal rats. The values obtained by the indirect method were shown to depend in great part upon the length of the constricting cuff applied to the tail.

**COMMENT.** These experiments demonstrate that elevation of the body temperature of normal or hypertensive rats is accompanied by an elevation of systolic and diastolic blood pressure and cardiac rate. When moderate elevation of temperature was maintained for prolonged period (2 hrs.), the blood pressure remained elevated. The more rapidly the body temperature was raised, the



more rapidly did the blood pressure rise. The amount of increase of blood pressure was roughly proportional to the elevation of temperature; gradual elevation of the temperature was accompanied by as great an increase of blood pressure as more rapid elevation.

Decrease of body temperatures as a result of cooling by an electric fan or induction of anesthesia was accompanied by a fall in blood pressure, that was succeeded by a secondary rise to a normal or hypertensive level.

Elevation of the body temperature made it possible to secure a higher percentage of unequivocal readings of blood pressure by the indirect method, but unfortunately also increased the blood pressure, thus making such readings difficult to evaluate. A rise in temperature occurred not only after exposure to external heat, but also following enclosing the rats in holders of sheet metal, presumably owing to the insulating effect of the solid walls. In holders made of wire mesh, only minimal elevation of temperature occurred.

When precautions were taken to avoid either increase or decrease of body temperature just preceding or during recording of blood pressure by the indirect method, the systolic pressures were in the range of 65 to 95 mm.Hg. It was necessary to maintain the temperature of the water in the plethysmographic chamber at 36.5° to 39°C. in order to overcome reflex vasoconstriction of the arteries of the tail. In our experience, levels of 130 mm.Hg or higher may be regarded as hypertensive for the rat when obtained under these conditions.

In the evaluation of procedures designed to change blood pressure, as from hypertensive to normal levels, it is suggested that utmost care be taken in the measurement of blood pressure lest extraneous factors such as changes in body temperature be permitted to exert their influence. This suggestion is made in view of the widespread practice of heating rats before measurement of blood pressure by the indirect method and of using anesthesia with its concomitant fall in body temperature before measurement of the intra-arterial method. The considerable variation in the range of normal blood pressures of rats and the inconsistent values obtained by both direct and indirect methods may be accounted for by failure to maintain body temperature at the normal level during the measurements.

From the data available it is impossible to obtain a full understanding of the mechanism underlying the changes in blood pressure. It has been recognized that elevation of the temperature of the body is associated with an increase of cardiac and basal metabolic rates as well as of the blood pressure of rabbits (2, 3) or human beings (2, 10). The increase of cardiac rate suggested an increase of cardiac output. An increase of basal metabolism may follow even brief exposures to external heat and contribute to increases of cardiac rate, blood pressure, and rectal temperature.

The changes in blood pressure were best correlated with changes in the cutaneous temperature; the rectal temperature followed more slowly. This parallelism suggests a cutaneous-vasomotor reaction in the initiation of the pressor and depressor effects. The secondary rise of the pressure after pro-

longed cooling in the presence of a declining rectal temperature may be attributed to peripheral vasoconstriction with increase of peripheral resistance.

A more simple explanation of the pressor effect may be the direct action of warmed blood upon the medullary vasomotor centers. It has been shown that heating the blood increases the heart rate (11) and elevation of the temperature of the blood above a critical level is a physiological stimulus for sweating (12). The initial marked rise in blood pressure, associated with even gradual elevation of rectal temperature may mean that when a critical level of cerebral temperature is exceeded circulatory adjustments, including a change in blood pressure, may follow. It is the early abrupt rise even with minimal heating, that constitutes the greater part of the pressor response.

The immediate depressing effect of general anesthesia (barbiturates) upon blood pressure of rats may be brought about by paresis of the temperature regulating center and depression of the metabolic rate leading to decreases of body temperature and cardiac output. Corcoran and Page (13) reported recently an increase of the blood pressure in dogs after barbiturate anesthesia. Their observations began 45 minutes after injection of the anesthetic and continued from 30 to 50 minutes. If our results in rats can be made applicable to dogs it is possible that an early depressing effect of the anesthesia preceded the reported rise.

The cooling effects in unanesthetized animals resemble those observed in anesthetized rats but the mechanism may be different. In the latter vasomotor control is more or less abolished. In the former, preliminary heating is required to demonstrate the effect of cooling and the declines are always from hypertensive levels. Not enough experiments have been done to say whether the secondary rise regularly reaches hypertensive levels in either type. The cause for the subsequent rise in blood pressure may be intense peripheral vasoconstriction or increased metabolic rate with its consequent increased cardiac output in response to the progressive loss of body heat.

#### SUMMARY

The relation between blood pressure, cardiac rate, and the temperature of the rat has been investigated. Indirect (plethysmographic) and direct (Hamilton's optical manometer) methods for measuring the blood pressure were used.

The systolic pressure of trained, normal unanesthetized rats, as measured by a modification of the indirect method in which there is no change in the rectal or cutaneous temperature, was 65 to 95 mm. Hg at room temperature (24° to 28°C.). To avoid the elevation of body temperature that occurs when animals are held in containers with solid walls, an animal holder with walls of wire mesh was found useful.

Elevating the cutaneous and rectal temperatures of normal anesthetized or unanesthetized rats was regularly followed by a progressive rise in blood pressure. The more rapidly the cutaneous temperature was increased, the more rapid was the blood pressure response. Fluctuations in cutaneous temperature corre-

sponded more closely in time and extent with the changes in blood pressure, than did the changes in rectal temperature.

Depressing the temperature of normal unanesthetized or anesthetized rats was followed by a fall of blood pressure; after 30 minutes or more the blood pressure rose again, occasionally to hypertensive levels.

Cardiac rate rose and fell with the blood pressure except in heat stroke when it remained accelerated after the pressor effect had disappeared.

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# AGE, SEX AND SPECIES VARIATIONS ON BLOOD PRESSURE IN NORMAL RATS<sup>1</sup>

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The authors studied correlations between age, sex, species and the arterial blood pressure in Wistar Albino and Gray Norway rats. The rat colony of the Wistar Institute was used, which contained a large series of rats covering the three year span of the rat's life. We have found no similar measurements in the literature.

**METHOD.** The method of Griffith (1) was used to measure blood pressure. The rat was maintained under a fixed and standard depth of ether anesthesia and arterioles in the skin of the hind foot observed for flow. A miniature blood pressure cuff (8-9 mm. in width and 2 cm. long) applied to the hind limb was inflated, and the pressure at which flow stopped was recorded as the systolic pressure. The final recorded blood pressure for a given rat was the average of four to seven successive readings by two observers taken from different vessels within a ten minute limit of anesthesia. Whenever such readings showed relatively poor agreement (4 out of 7 were required to fall within one blood pressure group), the rats were either retested at a later date or the reading entirely excluded. The mortality of the method was zero for young rats and about 2 per cent for very old ones.

**RESULTS.** A scatter diagram, with age as abscissa and blood pressure as ordinate, showed a positive linear relationship between the two variables until the upper age limits when the slope increased slightly and the curve was skewed upward. On the basis of a linear relationship the calculated line of regression was  $Y = 115.4 + 0.06X$ . Standard error of estimate ( $S_y$ ) was 36.6. The expected blood pressure in millimeters of any albino rat of a given age in days, therefore, equals  $115.4 + (0.06 \text{ times the number of days of age}) \pm 36.6$ . The coefficient of correlation ( $r$ ) for this relationship was 0.425 and the partial correlation coefficient, holding factor of sex constant, was 0.410. This gave a significance to the correlation well beyond the 1 per cent level.

In table 1 the number and per cent of albino rats falling within certain blood pressure ranges were compared in each of four age groups ranging from 110 to 910 days old. These arbitrary blood pressure ranges were:

- I—pressures less than 140 mm. of mercury.
- II—pressures between 140-159 mm. of mercury.
- III—pressures between 160-179 mm. of mercury.
- IV—pressures 180 mm. of mercury and above.

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In the youngest age group (110-140 days old) thirty-three rats (80 per cent of this age group), fell into blood pressure range I. This percentage of group I fell to 68 per cent (i.e., 37 rats) in the 450 to 500 day age group, to 41 per cent (i.e., 7 rats) in the 640 to 770 day age group, and to only 11 per cent (i.e., 2 rats) in the 825 to 910 day age group. The other ranges, II, III, IV, show a uniform increase in percentage of hypertensive rats with increasing age.

A correlation between sex and blood pressure was made. The coefficient of partial correlation, holding age factor constant, was only 0.025, which has no

TABLE 1  
*Age correlation with blood pressure in albino rats*

BLOOD PRESSURE GROUP	110-140 DAYS		455-500 DAYS		640-770 DAYS		825-910 DAYS	
	No.	%	No.	%	No.	%	No.	%
<i>mm. Hg</i>								
I. Less than 139.....	33	80.5	37	68.5	7	41.2	2	11.1
II. 140-159.....	3	7.3	4	7.4	5	29.4	6	33.3
III. 160-179.....	1	2.4	3	5.6	1	5.9	3	16.7
IV. Over 180.....	4	9.8	10	18.5	4	23.5	7	38.9
Totals.....	41	100.0	54	100.0	17	100.0	18	100.0

TABLE 2  
*Species correlation with blood pressure in Albino and Gray Norway rats*

BLOOD PRESSURE GROUP	100-200 DAYS OLD				350-900 DAYS OLD			
	Albino		Gray Norway		Albino		Gray Norway	
	No.	%	No.	%	No.	%	No.	%
I	33	80.5	8	66.6	46	51.6	14	66.6
II	3	7.3	3	25.0	15	16.9	3	14.4
III	1	2.4	0	0.0	7	7.9	2	9.5
IV	4	9.8	1	8.4	21	23.6	2	9.5
Totals.....	41	100.0	12	100.0	89	100.0	21	100.0

significance. There was, moreover, no significant difference between the *r*'s of age and the blood pressures in the male and female groups.

Table 2 shows the correlation between Gray Norway and Albino rats. These were divided into two similar age groups as indicated. There was no difference in the group 100 to 200 days. In the group 400 to 900 days, however, there was a slight trend (12 per cent) toward more hypertension in the albino. It is important to note that a greater number of older albinos were available for the study so that the grays in this comparison were somewhat younger. For this reason no significance should be attached to this correlation.

DISCUSSION. There are two sources of error in the method, namely, vascular spasm and anesthesia. A series of discordant consecutive readings usually was

due to vascular spasm, which is sometimes recognized by the appearance of the vessels themselves. It is felt that the maintenance of a constant and satisfactory depth of anesthesia reduces this factor to a minimum as a source of error.

The level of arterial tension in the rat is similar to that in man. Our results correspond to the general trend in man (2), namely, increasing incidence of hypertension with age. There is no record in the literature of blood pressure recordings in older rats (i.e., over 200 days). Our findings in the 110 to 140 day age group correspond with values found by Griffith (1, 3) and by Schroeder (4) in normal albino rats—namely, blood pressure levels in the range of 110 to 120 mm. of mercury.

No blood pressure differences between the two sexes were observed; nor was there a difference in the menopausal age group (450–600 days old) contrary to that which might have been anticipated from the findings in man (2).

The temperaments of the two species of rats were noted to be different. The Gray Norways are much less tame, become emotionally upset more readily. They exhibit other physiologic differences—for example, they are more susceptible to ether anesthesia—but there is no significant difference between the blood pressures of these two species.

#### SUMMARY

1. Blood pressures in a series of 130 Wistar Albino rats and 33 Wistar Gray Norways were determined.

2. The Wistar Albinos exhibit a definite tendency toward higher blood pressures with increasing age.

3. No sex differences in blood pressure were noted in the Wistar Albino rats.

4. There are no significant blood pressure differences between these two species.

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## BLOOD PRESSURE IN RATS SUBJECTED TO AUDIOGENIC STIMULATION

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With the recent emphasis on the humoral origin of hypertension, the neurogenic aspect has been less emphasized. Weiss in a recent article (1) discussed the psychosomatic phase of this problem and pointed out that many of the symptoms referable to hypertension were really the manifestations of a psychoneurotic conflict. In consequence, there is often no cause and effect relationship between the hypertension and the symptoms. According to Weiss, both the symptoms and the hypertension may be the results of the psychoneurosis. As an experimental approach to this problem, blood pressure measurements were made on rats subjected to audiogenic stimulation.

**METHOD.** Control and experimental groups of two species of rats, viz., Wistar Albino and Wistar Gray Norways, were studied. The experimental animals were those which Farris and Yaekel (2, 3, 4) had been subjecting to audiogenic stimulation since the time of weaning; i.e., twenty-one days of age. These animals had been exposed to a constant air blast every week-day for from five to ten minutes per day up to the time our measurements were made. Control animals were litter mates, raised and treated identically except for the stimulations and the consequent lesser frequency of daily handling. The controls were kept so that this second group did not hear the air blast.

Rats subjected to this air-blast reacted in varying degrees. Almost all showed dilated pupils, increased frequency of urination and defecation. What was arbitrarily called a "reaction" consisted of a running attack in which the rat ran and leaped rapidly about the pen. Usually, but not always, this attack was terminated by a series of clonic convulsions and a final transient (3-5 min.) state of tonic rigidity often so marked that the extremities were able to be molded into bizarre positions. Rarely did a rat die following one of these attacks.

Detailed record was kept of the daily response to stimulation of each experimental rat, and at any day, the reaction score of each could be calculated on a percentage basis of the number of "reactions" shown compared to the total number of times the rat had been stimulated.

Blood pressure measurements were made on these animals by the method of Griffith (5). Practically all readings were taken between 7:00 p.m. and 11:00 p.m. Experimental rats were alternated with litter mate controls in the same evening to minimize the effects that daily temperature and humidity changes might have on anesthesia and, consequently, on blood pressure.

**RESULTS.** Blood pressure readings were recorded as falling into one of four groups:

Group I—pressures less than 140 mm. of mercury

Group II—pressures between 140–159 mm. of mercury

Group III—pressures between 160–179 mm. of mercury

Group IV—pressures above 180 mm. of mercury

In order to facilitate rapidity in the procedure and shorten the duration of anesthesia, no attempt was made to read the blood pressure any more accurately than to be positive that the reading fell definitely into one group or the other. Any blood pressure 160 mm. or over (groups III and IV) was considered hypertensive.

The Gray Norways were studied as two groups differing in age. The younger contained twenty-four rats of ages ranging from 130 to 205 days, the older, thirty-nine rats, from 340 to 890 days old. It can be seen from table 1 that there was no blood pressure difference between experimentals and controls in the younger group. The 8.4 per cent hypertension in the control group as compared with the absence of hypertension in the experimental group involves a difference of only one rat.

TABLE 1

*Distribution of hypertension among control and experimental Gray Norway rats*

BLOOD PRESSURE GROUP	130-205 DAYS OLD				340-890 DAYS OLD			
	Control		Experimental		Control		Experimental	
	No.	%	No.	%	No.	%	No.	%
I	8	66.6	12	100.0	14	66.6	6	33.3
II	3	25.0	0	0.0	3	14.4	1	5.6
III	0	00.0	0	0.0	2	9.5	1	5.5
IV	1	8.4	0	0.0	2	9.5	10	55.6
Total.....	12	100.0	12	100.0	21	100.0	18	100.0

In the older group, however, four out of twenty-one animals, or 19 per cent, of the control group were hypertensive, while eleven of nineteen, or 61 per cent, of the experimental animals were hypertensive. These figures, when subjected to chi square analysis, give a probability of less than 1 in 100 that this difference is due to chance.

When the percent frequency of reaction to audiogenic stimulation is correlated with blood pressure in the experimental group, a remarkable correlation is found. A rat is considered a good "reactor" when it develops a full blown audiogenic seizure (i.e., running and convulsive attacks) seven out of ten times it is stimulated. This 70 per cent figure though arbitrary is really unimportant because in actual practice the reactors are usually well above this figure and the non-reactors well below it, as shown in table 2. Of the eleven hypertensive rats in the older experimental group, nine were reactors. Of the other two hypertensives one had a reaction score of 57.2 per cent; the other, a group IV hypertensive, was only a 22.6 per cent reactor. Of the seven normotensives in this older experimental group, all were poor reactors.

In the younger group of Grays there were no hypertensives, but there were



four excellent reactors and two with scores (60 and 48 per cent) which might easily be improved in the ensuing two hundred days. Measurements on this younger group are to be repeated when it reaches approximately the four hundred day old range.

Little can be learned from the albinos because the series of experimentals is too small and inadequately controlled.

TABLE 2  
*Experimental Gray Norways with reaction score and blood pressure itemized*

DAYS OLD	IDENTIFICATION NUMBER	BLOOD PRESSURE GROUP	SEX	REACTION SCORE
				%
367	142	I	F	62.1
387	134	I	F	14.6
387	135	I	F	17.3
340	143	IV	M	84.1
340	151	IV	F	98.6
340	148	IV	F	96.4
340	147	IV	M	77.6
340	145	IV	M	97.8
340	150	IV	F	96.3
828	60	IV	M	78.1
603	105	IV	M	22.6
762	31	IV	F	73.6
686	84	IV	M	72.3
610	97	III	M	57.2
393	124	II	M	37.1
393	127	I	F	50.6
381	121	I	F	29.5
405	136	I	F	18.0
139	173	I	M	85.2
136	164	I	M	76.5
134	159	I	M	35.8
134	158	I	M	7.4
136	163	I	M	27.2
140	156	I	M	89.4
169	189	I	F	2.9
199	175	I	F	25.9
169	192	I	F	34.8
203	157	I	F	78.8
205	154	I	M	60.0
205	169	I	M	48.1

DISCUSSION. Systolic pressure only was measured in this study. It is realized that diastolic readings also would have been of greater value as a criterion for hypertension. Moreover, blood pressure was used as the only criterion for hypertension. Schroeder (6) who, in a blood pressure study on rats, used also cardiac size, classed those rats as hypertensive which have either hypertension and cardiac enlargement or cardiac enlargement alone. An animal with hyper-

tension and no cardiac enlargement was considered to have "anesthetic hypertension". He used a certain dose of barbiturate per gram of body weight as anesthetic agent and had poor control of depth as attested by the 20 per cent mortality of his method. Moreover, the incidence of hypertension without cardiac hypertrophy—or "anesthetic hypertension"—is small, only 6 to 7 per cent. Because of adequate control of anesthetic level and close controls for the experimental group in the present study "anesthetic hypertension" has been disregarded.

Much has been written on audiogenic seizures in Wistar rats (2, 3, 4). The central nervous system pathways involved in this reaction have not been worked out as yet. Certainly the auditory nerve must be the first neuron in the chain. Whether the cerebral cortex is required to effect the reaction is not known. It is presumed that the mechanism occurs through the medium of the central nervous system and autonomic pathways, though this is by no means proven. One cannot say that this audiogenic stimulation has caused hypertension. It can be concluded from this result, however, that the rat who reacts most vigor-

TABLE 3

*Distribution of hypertension among control and experimental Wistar Albino rats*

BLOOD PRESSURE GROUP	CONTROL		EXPERIMENTAL	
	No.	%	No.	%
I	13	54.2	3	23.0
II	5	20.8	2	15.4
III	1	4.2	1	7.7
IV	5	20.8	7	54.0
Total.....	24	100.0	13	100.0

ously and consistently to audiogenic stimulation develops hypertension. Thus, audiogenic seizures may be simply a measure of that factor in the animal's constitution ("nervous tone") which causes the hypertension. Though this work by no means proves the neurogenic origin of hypertension, it is definite objective evidence that one type of experimental nervous stimulation, at least, is associated with increased tendency to hypertension in the rat.

It is evident that one cannot gain any insight as to familial incidence of hypertension in these animals from our figures as presented. There was, however, a markedly hypertensive litter of nine rats in our series. Six of these nine were experimentals and were all hypertensives and all excellent reactors. The other three were controls, and only one of these was normotensive. Naturally, having six of our eleven hypertensive experimental animals in a single litter makes it less likely that the difference between experimental and control groups is significant and due to audiogenic stimulation. This fact does not detract from the correlation between reactors and hypertensives. It will be interesting to see whether the excellent reactors at 150 to 200 days of age develop hypertension by the time they reach the 400-day old range.

Histologic or grossly anatomic study of these rats was not done. The possibility exists that degenerative changes in the brain might have occurred from repeated convulsions in the good reactors and that these have led somehow to hypertensive changes in the vascular system.

In regard to the Albino rat, nothing was able to be concluded definitely because of an inadequate series. These animals are poor reactors to the audiogenic stimulus. Any animal which reacts does so usually only up to 150 days of age and then stops, giving a poor score after reaching 400 to 600 days of age. Thus, we were not able to correlate reactors with hypertension in this group of animals.

Blood pressure group				
HYPOREACTORS	HYPERREACTORS		HYPOREACTORS	HYPERREACTORS
•   •   • •   •   • •   •   •	•   • •   •	Less than 139 mm.	•   •   • •   •   •	—
—	—	140-159 mm.	•	—
—	—	160-179 mm.	•	—
—	—	180+ mm.	•	•   •   • •   •   • •   •   •
130-205 day old group			340-830 day old group	

Fig. 1. Correlation of blood pressure with the reaction score to audiogenic stimulation.

#### SUMMARY

1. Blood pressures were taken in 18 experimental rats subjected to daily audiogenic stimulation and 21 controls at 400 to 900 days of age; and on 12 controls and 12 experimentals between 130 to 200 days of age.

2. No difference in incidence of hypertension was found in the younger group, but a significant difference was found in the older group—there being 61 per cent hypertensives among the experimentals and 19 per cent among the controls.

3. Of the eleven hypertensive groups in older experimental group, nine were excellent reactors to audiogenic stimulation. Of the seven normotensives in this group, none were good reactors.

4. We believe this to be objective evidence for neurogenic influences in the etiology of hypertension.

*Addendum.* Further determinations of blood pressure on twelve control and sixteen air blasted Gray Norway rats at 487 to 565 days of age have borne out

the above results. The composite results show that only five out of thirty-nine controls but twenty out of thirty-four experimentals were hypertensive.

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# SHOCK PRODUCED IN DOGS BY HYDATID FLUID

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The toxicity of the fluid of cysts of *Echinococcus granulosus* has been repeatedly reported (1, 2, 3). Giusti and Hug (3) have shown that hydatid fluid produces severe to fatal shock when injected intravenously into dogs, cats, guinea pigs and rabbits. In the dog the symptoms were somewhat similar to those of anaphylactic shock, since there was a fall of carotid blood pressure, with tachyphylaxis, leukopenia and incoagulability of the blood. In man accidents produced by the rupture of hydatid cysts have been reported in several instances (4, 5). Those accidents are usually attributed to phenomena of sensitization to foreign antigens present in hydatid cysts. The rupture of the cysts would bring the system into contact with massive doses of substances to which the organism had been progressively sensitized and anaphylactic accidents would follow combination of the antigens with the respective antibodies. This hypothesis, however, might be advantageously enlarged in consideration of the fact that severe to fatal shock develops among normal dogs after a *first* injection of hydatid fluid. The fluid appears to contain a substance which is primarily toxic for dogs, as shown by previous investigators and confirmed in the present paper.

One of us, in collaboration with Recarte and Balea (6), has shown an increase of the histamine content of the blood following injection of hydatid fluid into the veins of dogs and has assumed a certain relationship between the release of histamine from the tissues and the fall of carotid blood pressure which is a constant symptom following administration of hydatid fluid. The experiments reported in the present paper were done with the aim of verifying how far this release of histamine might be concerned in the outcome of the shock produced in dogs by the injection of hydatid fluid.

**MATERIAL AND METHODS.** The hydatid fluids used in our experiments were from three sources: sheep, calf and pig. In some of the experiments a pooled sample of the three fluids was used since it has been verified that only minor differences can be ascribed to each one of the fluids employed.

Unless otherwise stated, the dogs were anesthetized with choralose (0.1 gram per kgm. of body weight) in 1 per cent solution in hot saline. This solution was injected through a femoral vein, immediately after being cooled to body temperature. The estimations of histamine in the samples of blood were done by Code's method (7). In several instances the Barsoum and Gaddum method (8) was applied in view of the fact that besides histamine Anrep, Barsoum, Talaat and Wieniger (9) described a substance in Code's extracts of dog's blood which contracts smooth musculature and can be excluded by four extractions of the dry residue with alcohol. In our experiments, however, we could not find any

substantial differences between Code's and Barsoum and Gaddum's extracts for histamine, as tested on the guinea pig gut. In a few experiments the fragments of the liver were also extracted by both methods and the results agreed closely.

In the experiments in which the histamine in the liver was estimated before and after shock, a small fragment of the organ was quickly removed with a sharp knife and the abdominal wall was closed with hemostats until the end of the experiment, when a second fragment was excised from another lobe of the liver.

The perfusions of the liver were carried out in the following way: the dog was anesthetized with chloralose, a cannula was inserted into the carotid artery and the abdomen was widely opened from pubis to sternum; the trunk of the portal vein was exposed and two ligatures were put in place; the animal was exsanguinated through the carotid cannula and just before death the perfusing cannula was inserted into the trunk of the portal vein, all the collateral rami of the portal vein distal to the cannula were tied and the left lobes of the liver, as well as the gallbladder, were tied off and excised; the liver was freed from all connections with the abdominal organs and diaphragm and transferred to an appropriate mould of paraffin.

**RESULTS.** The injection of 30 to 50 cc. of hydatid fluid causes a slow fall of carotid blood pressure, as shown in figure 1. After partial recovery, the reinjection of the same dose of hydatid fluid is entirely ineffective. This fact shows that after a first response the dogs become desensitized to further injections of the same substance. This tachyphylactic effect suggests that the shock produced by hydatid fluid is mediated by some agent which becomes exhausted at the second injection. Another interesting fact that suggests that the shock resulting in dogs from injection of hydatid fluid is produced indirectly through the liberation of biologically active substances is the decrease of the coagulability of the blood that frequently accompanies the severe fall of the carotid blood pressure. In a few cases the shed blood remained fluid for twenty-four hours.

If the hydatid fluid is concentrated ten times at boiling temperature a sharper decline of the arterial blood pressure is observed, preceded by a small evanescent fall. This first evanescent fall of the carotid blood pressure can be ascribed to the presence of histamine in the hydatid fluid, since prolonged dialysis abolishes this effect.

That the substance producing shock is not destroyed by heat nor dialyzable through cellophane is shown in the experiment presented in figure 2. The hydatid fluid for this experiment was dialyzed against distilled water during forty-eight hours and the volume was reduced to a fifth by prolonged boiling in open air. Severe shock followed the injection of 10 cc. of the concentrate, corresponding to 50 cc. of hydatid fluid.

A further knowledge of the properties of the substance producing shock was secured in an attempt to obtain it in a purified form. The experiment shown in figure 3 was performed with a highly purified active material (DAI), obtained from hydatid fluid according to the following procedure: to 100 cc. of the fluid was added 5 grams of trichloroacetic acid and the mixture was kept overnight in

the icebox; the next day, the material was filtered clear through paper (after the addition of a small amount of Super-cell); the filtrate was transferred to cellophane bags and dialyzed against distilled water for forty-eight hours; the material was then concentrated in vacuo to a small volume and precipitated with 10

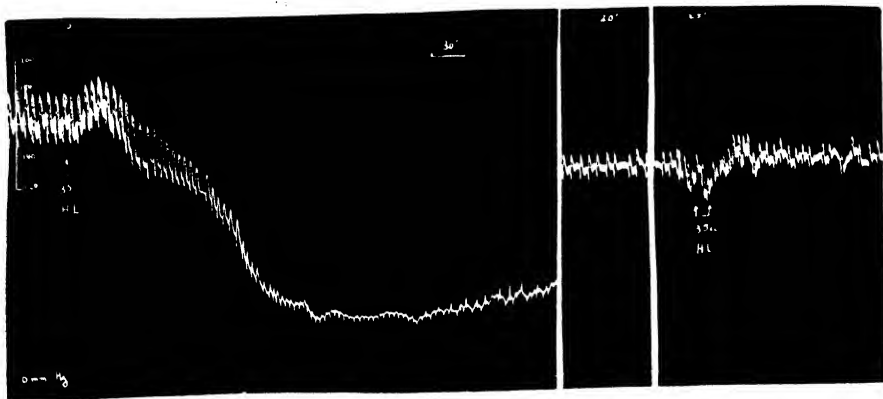


Fig. 1. Dog 3, 15 kgm. body weight. At the first arrow, 35 cc. of hydatid fluid were injected intravenously. Twenty-five minutes later, after partial recovery, the same dose of hydatid fluid was again injected without any appreciable effect on the carotid blood pressure.

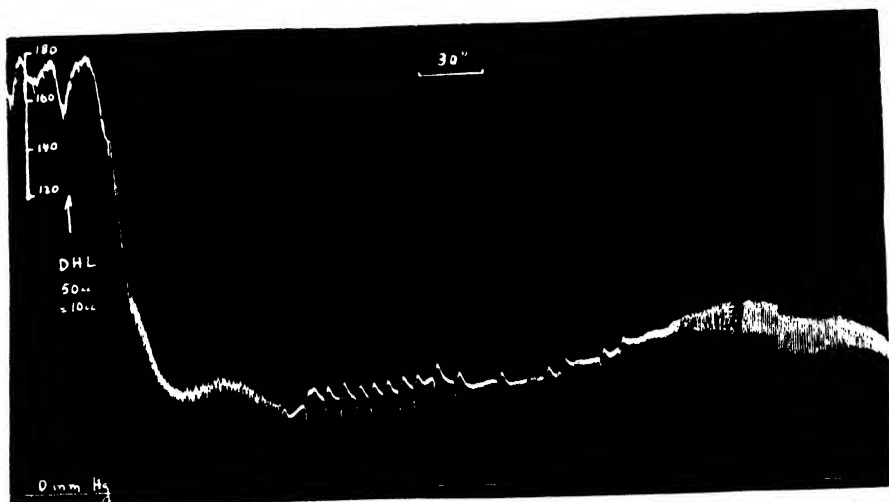


Fig. 2. Dog 9, 10.3 kgm. body weight. Ten cubic centimeters of dialyzed hydatid fluid (concentrated five times) were injected into the femoral vein. Note the sharp fall of carotid blood pressure, contrasting with the somewhat slower fall observed in dog 3 (fig. 1), which received a great volume of unconcentrated fluid.

volumes of acetone; the precipitate was collected, dried (yielding about 60 mgm. of solid) and dissolved in 10 cc. of saline solution. This solution produced a very sharp fall of the carotid blood pressure, as shown in figure 3.

Samples of blood were taken from sixteen dogs before and after the intravenous

injection of hydatid fluid or of products derived from it and the total concentration of histamine in the blood was estimated by Code's procedure. As shown in table 1 and figure 4, in several cases there was a drop of the concentration of histamine in the blood after injection of the hydatid fluid. In dogs 4, 5, 10 and 12 the increase of the histamine content was indisputable and was early enough to have some significance for the production of the shock. In dogs 2, 6, 7 and 9 there was a late increase of the histamine content that could be observed only when the carotid blood pressure was already increasing. All those facts point to the conclusion that the increase of histamine in the blood does not explain the precipitous fall of the carotid blood pressure. That a certain release of histamine accompanies the shock is made very probable from data obtained by estimating the concentration of histamine in the liver before and after the injection of hydatid fluid. In four of six cases there was a definite decrease of the histamine

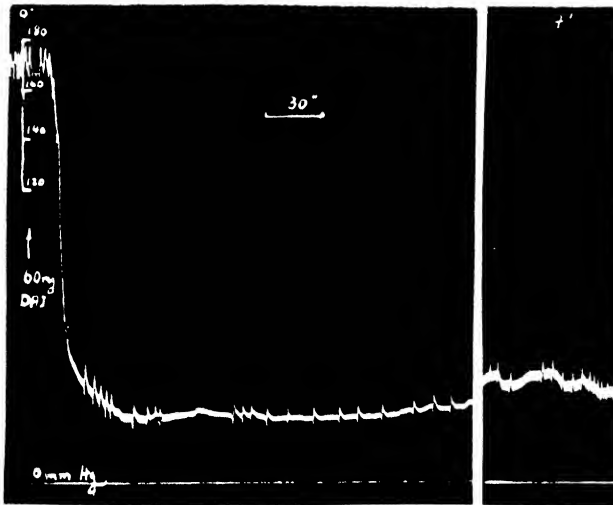


Fig. 3. Dog 10, 8.5 kgm. body weight. Sixty milligrams of the dry substance (DAI) obtained from 100 cc. of hydatid fluid were injected.

content of the liver. But even here there was no parallelism between the decrease of the concentration of histamine in the liver and the intensity of the shock. In dogs 3 and 12 the injection of hydatid fluid produced moderate to severe shock, although the histamine content of the liver changed very slightly or not at all.

The livers of four dogs were perfused with Tyrode's solution and 10 cc. of concentrated (10:1) hydatid fluid was injected through the perfusing cannula. Only small amounts of histamine (2 to 4 micrograms, total) were liberated in those conditions and in no case was there a liberation of the amounts which might be expected from the experiments *in vivo*. The possibility that a substance present in blood might contribute to the release *in vivo* of histamine from the liver induced us to add defibrinated blood to the perfusing fluid. Even so, the injection of hydatid fluid did not lead to any release of histamine in significant amounts.



TABLE 1

*Histamine in the blood of dogs given hydatid fluid or substances prepared from it*

DOG	WEIGHT OF DOG	ANESTHETIC AGENT USED	MATERIAL INJECTED	BLOOD HISTAMINE, MICROGRAM PER CUBIC CENTIMETER			LIVER HISTAMINE, MICROGRAMS PER GRAM	
				Before	After		Before	After
	kgm.				2 min.	5 to 10 min.		
1	9.0	Morphine, dial	40 cc. HL*	0.06	0.05	0.02		
2	9.5	Morphine, dial	35 cc. HL	0.10	0.10	0.13		
3	15.0	Chloralose	35 cc. HL	0.14	0.08	0.06	46.0	48.0
4	17.5	Chloralose	10 cc. CHL	0.12	0.20	0.13	52.0	29.0
5	11.5	None	10 cc. CHL	0.06	0.55	0.34		
6	10.0	Chloralose	10 cc. CHL	0.11	0.10	0.13		
7	10.0	Dial, ether	10 cc. CHL	0.05	0.04	0.08	15.0	10.2
8	8.0	Chloralose	10 cc. CHL'	0.13	0.06	0.07		
9	10.3	Chloralose	10 cc. CHL'	0.09		0.20		
10	8.5	Chloralose	60 mgm. DAI	0.06	0.13	0.06		
11	13.0	Chloralose	1 gram SHL	0.09	0.07	0.09	36.9	27.4
12	6.5	Chloralose	3 grams SHL	0.06	0.09	0.11	24.0	24.0
13	9.7	None	1 gram SHL	0.13	0.09	0.09		
14	6.5	None	1 gram SHL	0.09	0.02	0.04		
15	13.4	Chloralose	3 grams SHL	0.12	0.12			
16	11.0	Chloralose	3 grams SHL	0.14	0.15		12.3	6.4

\* HL signifies the hydatid fluid without any treatment; CHL means ten times concentrated hydatid fluid; CHL' means five times concentrated hydatid fluid; DAI represents the substance which has been extracted from hydatid fluid as indicated in the text; SHL means the solid material obtained from 3 liters of pooled hydatid fluid submitted to concentration *in vacuo* at 60° to 80°C.: each gram corresponds approximately to 100 cc. of the original fluid.

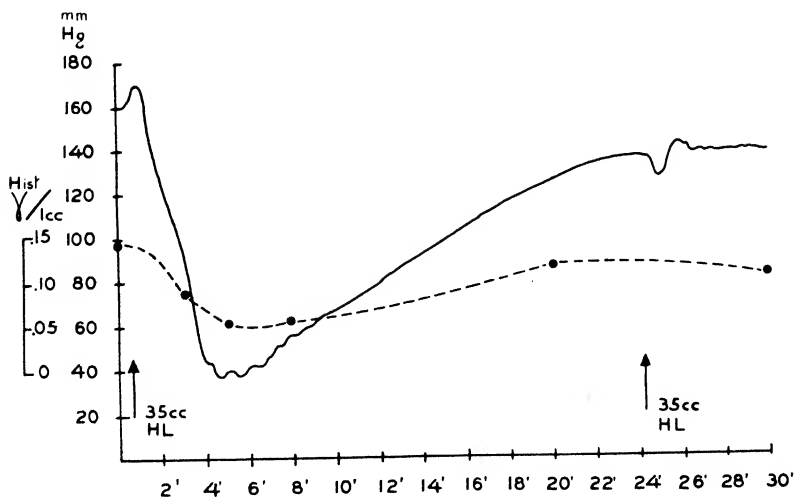


Fig. 4. Dog 3. Histamine content of the blood samples taken at several intervals before and after the injection of hydatid fluid. There was a definite fall of the blood histamine which attained its lowest level when the carotid blood pressure was at a minimum. Dotted line: histamine content of total blood. Full line: carotid blood pressure.

In one experiment, the lung of another dog was perfused with Tyrode's solution and the concentrated hydatid fluid was injected after one hour of perfusion. There was a total release of not more than 4 micrograms of histamine during a forty minute perfusion. This definitely shows that hydatid fluid is unable to release significant amounts of histamine either from the liver or from the lungs of dogs.

In no case has there been any indication of the release of a slowly reacting substance (S.R.S.) as described by Feldberg and Kellaway (10) in their experiments on snake and bee venoms.

COMMENT. From the foregoing results it is apparent that liberation of histamine cannot account for the shock induced in dogs by the injection of hydatid fluid. That the shock is produced indirectly is suggested by the tachyphylactic effect and by the fact that in several instances the blood becomes less coagulable after the injection of hydatid fluid than before its injection. As those occurrences are common features of shock produced by peptone, trypsin and anaphylaxis, it is interesting to compare the results referred to previously with those that have been described in the literature dealing with the mechanism of the aforementioned types of shocks. That histamine is released from the liver of dogs in amounts that might explain anaphylactic shock was shown in the experiments of Ojers, Holmes and Dragstedt (11). A substance indistinguishable from histamine appears in the peripheral blood of sensitized dogs in amounts which might explain the fall of carotid blood pressure, as shown by Dragstedt and Gebauer-Fuelnegg (12) and Code (13). In the cases of trypsin and peptone shocks, similar results have been described by one of us (14), Ramirez de Arellano, Lawton and Dragstedt (15), Holmes, Ojers and Dragstedt (16) and Dragstedt and Mead (17).

In the case of the shock produced by hydatid fluid there is frequently rather a decrease of the total blood histamine and only in the late stages is there sometimes a small increase of the histamine content of venous blood, this increase occurring frequently when the carotid blood pressure had begun to increase and recovery was manifest. In anaphylactic shock, there is a definite relationship between the extent of the fall of carotid blood pressure and the decrease of the concentration of histamine in the liver, as shown by Ojers, Holmes and Dragstedt (11). After injection of hydatid fluid the decrease of the concentration of histamine in the liver is small in most cases and does not bear any relationship to the severity of the shock. Perfusion of the liver and lungs of dogs did not lead to any appreciable release of histamine. All these facts point to the conclusion that the release of histamine is not seriously concerned with the production of shock in dogs following the injection of hydatid fluid. Consequently the decrease of the concentration of histamine in the liver as observed in experiments *in vivo* might be the consequence of the shock rather than its cause. Especially, the bad nutrition of hepatic cells following the fall of blood pressure might account for some injury to tissue and consequent release of histamine, which appears in small amounts in the circulating blood.

If it is easy to rule out histamine as the cause of the fall of systemic pressure

produced by hydatid fluid, it is more difficult to advance any suggestion on the nature of the mediator of this kind of shock. Liberation of heparin might explain the evanescent increase of the clotting time of the blood as observed in a few experiments, although further experimentation is necessary to settle this point. There was no indication of a liberation of a slowly reacting substance (S.R.S.) in the experiments on perfusion of the liver and lungs of dogs with hydatid fluid. Snake and bee venoms liberate this substance, as shown by Feldberg and Kellaway (10). Kellaway and Trethewie (18) have shown a release of a slowly reacting substance and of adenylyl compounds in anaphylactic shock. Trypsin also was shown by Trethewie (19) to release S.R.S. and adenylyl compounds from the perfused liver of rabbits. As S.R.S. does not probably play any rôle in the shock produced by hydatid fluid, there remains the possibility that adenylyl compounds which are hypotensive and resistant to atropine might be released by hydatid fluid from the tissues of normal dogs. This possibility, however, deserves direct experimentation that has not yet been performed. Interesting enough are the facts described by Kellaway and his associate (20, 21) on the possibility of a sort of injury of tissue without liberation of histamine. The toxin of *Clostridium perfringens* type D liberates histamine, slowly reacting substance and adenylyl compounds, while the toxin of *Clostridium perfringens* type A does not liberate histamine nor S.R.S. but liberates adenylyl compounds from the liver of the rabbit (20). The toxin of *Clostridium perfringens* type B liberates histamine from the lungs of cats but not from those of guinea pigs, while the toxin of *Clostridium perfringens* type C liberates S.R.S. but not histamine (21). Consequently there remains the possibility that the hydatid fluid produces a sort of injury of tissue which leads to a release, not of histamine or S.R.S., but of an unidentified shock-producing substance which might be the pharmacologic mediator of its toxic effect on dogs.

The decrease of the concentration of histamine in the blood might be explained by the leukopenia which follows the injection of the hydatid fluid, as shown by others and confirmed by ourselves, since Code (22) has shown that histamine in the blood of dogs is mostly confined to the leukocytes.

#### CONCLUSIONS

Hydatid fluids from sheep, calf and pig produce shock when injected intravenously into dogs. After recovery, the reinjection of the same dose is ineffective (tachyphylaxis). During shock, a decrease of the coagulability of the blood was frequently observed.

Boiling in the open air and prolonged dialysis do not destroy the capacity of hydatid fluid to produce shock in the dog. Deproteinization with trichloroacetic acid and prolonged dialysis to remove the acid do not alter the capacity of hydatid fluid to produce shock. From those experiments, a method was devised to extract from hydatid fluid an active dry material which produced severe shock in the dog, when injected in the dose of 60 mgm.

Quantitative studies were made of the histamine content of the blood of dogs before and after the intravenous administration of hydatid fluid. In some cases

there was a decrease, in some an early increase and in others a late increase of the concentration of histamine in the blood. There was no apparent relationship between the concentration of histamine in the blood and the degree of fall of carotid blood pressure. The decrease of the concentration of histamine in the liver that was verified in several cases did not show any relationship to the severity of the shock. Experiments on the perfusion of the liver and lungs of dogs with hydatid fluid showed that the fluid is not capable of liberating histamine from the tissues of dogs.

From the foregoing findings, the general conclusion is drawn that histamine is apparently not concerned with this type of shock. The possibility of an unidentified substance being the mediator of the shock produced in dogs by hydatid fluid is discussed.

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# EFFECT OF HYDATID FLUID ON HISTAMINE CONTENT OF RABBIT BLOOD

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In the preceding paper (1) we have studied the mechanism of the shock produced in dogs by an intravenous injection of hydatid fluid. In several cases there was a marked decrease of the concentration of histamine in the liver, as estimated in fragments taken before and after the injection of hydatid fluid. Since there was no relationship between this decrease of the concentration of histamine in the liver and the severity of shock and since hydatid fluid was unable to liberate free histamine from the isolated liver of dogs, the decrease of the histamine content of the liver was assumed to be of minor significance in the explanation of the production of shock. In rabbits there is a fall of blood histamine when shock-producing substances (antigen, trypsin and peptone) are injected intravenously (2, 3, 4). In this sense, rabbit blood behaves like dog liver toward the aforementioned agents and it was interesting to verify the reaction of the rabbit to the intravenous injection of hydatid fluid. In contradistinction to what occurs in dogs, hydatid fluid does not produce shock in anesthetized rabbits and only minor symptoms follow the injection of this substance into non-anesthetized animals. This result is in disagreement with many reports pointing to the toxicity of hydatid fluid when injected intravenously into rabbits (5, 6). Interestingly enough, the blood histamine dropped sharply in almost all animals assayed in the experiments presented in the present paper.

The facts presented in this paper and in the preceding one show that a considerable decrease of the histamine content of dog liver and rabbit blood might have no relationship to the shock-producing effect of a substance.

**MATERIAL AND METHODS.** The hydatid fluids used in the experiments presented in this paper were from the same sources as those used in the experiments of the preceding paper: sheep, calf and pig. The three fluids are indicated as HL<sub>1</sub>, HL<sub>2</sub> and HL<sub>3</sub> in the experiments described subsequently. When the material used is simply indicated as HL, this means that a pooled sample was used. The treatments (dialysis, deproteinization with trichloroacetic acid, precipitation and extraction with solvents) to which the fluids were submitted during the experiments are indicated in the text.

The experiments on the ileum of the guinea pig were performed in a Dale apparatus with a perfusing bath of 3 to 4 cc. capacity. The histamine content of hydatid fluid was estimated either by adding the fluid directly to the bath or after extraction, following Code's procedure (7). For convenience, the histamine that has been estimated without extraction is called "free" histamine, although we cannot be sure whether the bound histamine (histamine "precursor") present

in hydatid fluid is capable of partially displaying some of the effects of free histamine.

Dialysis was performed in cellophane bags against distilled water. The external water (dialysate) was changed twice a day. In the cases in which the dialysate was tested on the guinea-pig gut, the volume was reduced in vacuo at 60° to 80°C. and the final volume was referred to the original volume of hydatid fluid before dialysis.

The concentration of histamine in rabbit blood was estimated by Code's method in duplicate samples of 1 cc. of total blood collected from the veins of the ear in syringes coated with petrolatum and containing 0.2 cc. of sodium oxalate (tenth-molar) for each 1.8 cc. of blood. In vitro experiments were performed according to the method described previously (6): two samples of 4 to 5 cc. of oxalated blood were put into contact with 1 cc. of saline solution and 1 cc. of hydatid fluid respectively; after three to five minutes' incubation at 38°C., the samples were centrifuged, the plasmas carefully collected and duplicate samples of 1 cc. taken up for extraction of histamine using Code's procedure. The substitution of sodium oxalate for the heparin was found satisfactory.

For blood pressure recording, the rabbits were anesthetized with urethane (1 gram per kgm. intraperitoneally). In one case, dial "liquid" (Ciba) and ether were used. Platelet counts were made following Fonio's procedure.

**RESULTS.** *Free histamine and the histamine "precursor" present in hydatid fluid.* The hydatid fluids contract the guinea-pig gut when tested directly by addition to the perfusing bath. This contracting effect was assumed to depend on the presence of free histamine, since arginine, which inhibits the histamine effects, also inhibits the contracting effect of the hydatid fluid. Calculated as histamine, this substance, which contracts the guinea-pig gut, is present in amounts which vary from 0.06 to 0.13 microgram per cubic centimeter of fluid. Submitted to Code's method of extraction, however, the hydatid fluids appeared to contain three to four times as much free histamine as that previously present in them (table 1). This difference was shown to depend on liberation of histamine from an inactive "precursor," since the fluid itself does not display any inhibitory effect on the histamine standard solution (fig. 1).

From table 1 it is apparent that histamine is not bound to proteins, since the inactive "precursor" passes slowly through cellophane. After twenty hours' dialysis, the hydatid fluid is almost entirely exhausted of its free or bound histamine. The experiments presented in figure 1 show graphically the facts mentioned previously. The histamine present in hydatid fluid can be found quantitatively in the first and second dialysates, while the material contained in the cellophane bags ( $E_{III}$ ) is entirely exhausted of its histamine activity after forty-eight hours' dialysis.

The experiments referred to in table 2 were carried on to test further the properties of this inactive histamine "precursor." The dry residues of samples of the hydatid fluids were submitted to extractions by several organic solvents such as alcohol, ether, acetone and chloroform. From those experiments it became apparent that the histamine "precursor" present in hydatid fluid can be fairly

well extracted with alcohol but that it is entirely insoluble in ether, chloroform or acetone.

TABLE 1  
*Free and bound histamine present in hydatid fluid*

NATURE OF THE EXPERIMENTS	HISTAMINE CONTENT, MICROGRAM PER CUBIC CENTIMETER		
	HL <sub>1</sub>	HL <sub>2</sub>	HL <sub>3</sub>
Hydatid fluid, tested directly.....	0.06	0.13	0.09
Hydatid fluid, submitted to Code's extraction....	0.20	0.40	0.30
Dialyzed hydatid fluid (HL <sub>1</sub> )*			
6 hours' dialysis.....	0.15		
20 hours' dialysis.....	0.03		
48 hours' dialysis.....	0		
Dialyzed hydatid fluid (HL <sub>2</sub> )*			
96 hours' dialysis.....		0	

\* The dialyzed fluids have been submitted to Code's method for extraction of histamine.

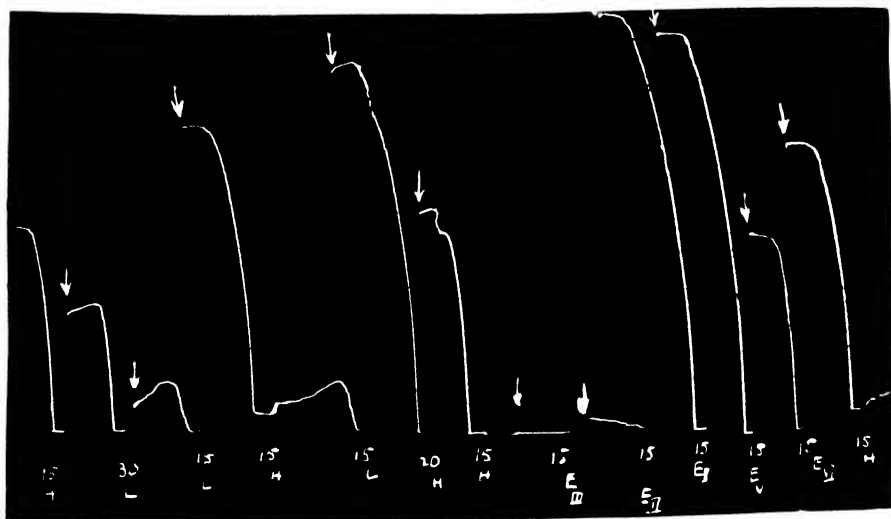


Fig. 1. Guinea-pig ileum. *H* = histamine base 1:5,000,000. *L* = hydatid fluid in nature. *E<sub>v</sub>* = hydatid fluid submitted to Code's method. *E<sub>I</sub>* and *E<sub>II</sub>* mean first and second dialysis water, concentrated to the original volume of the hydatid fluid. *E<sub>III</sub>* = forty-eight hours' dialyzed hydatid fluid, submitted to Code's method. Upper arrows indicate the change of the Tyrode solution in the perfusing bath. Addition of 0.15 cc. of hydatid fluid did not change appreciably the response to 0.15 cc. of histamine solution added without washing with new Tyrode solution. (All the figures represent hundredths of a cubic centimeter added to the perfusing bath.)

From the foregoing experiments one might conclude that hydatid fluid contains histamine in a bound condition and that the histamine can be set free through Code's method of extraction. This histamine "precursor" is a low

molecular complex, soluble in alcohol but practically insoluble in ether, acetone or chloroform.

*Experiments on rabbits.* The foregoing experiments have shown that it is possible to have a hydatid fluid entirely free of histamine either by prolonged dialysis against distilled water or by alcohol extraction of the dry residue obtained through evaporation in vacuo. In the preceding paper (1) we have also shown that after prolonged dialysis hydatid fluid does not lose its capacity to produce shock in dogs. In the rabbit the hydatid fluid when injected intravenously produced a fall of the histamine content of the blood. In the experiments presented in table 3, we have shown that this ability to produce a reduction of the total blood histamine is maintained after dialysis, deproteinization with trichloroacetic acid and precipitation of the concentrated material with acetone. The material used in these experiments was a pooled sample of the three hydatid fluids available.

TABLE 2

*Experiments on the properties of histamine "precursor"*

Samples of 20 cc. of hydatid fluid were evaporated under reduced pressure to complete dryness and each one was extracted with the indicated solvents. The residues and the extracts were again evaporated in vacuo and submitted to Code's extraction.

NATURE OF THE EXPERIMENTS	HISTAMINE CONTENT, MICROGRAM PER CUBIC CENTIMETER	NATURE OF THE EXPERIMENTS	HISTAMINE CONTENT, MICROGRAM PER CUBIC CENTIMETER
Alcohol extract.....	0.17	Acetone extract.....	0
Residue.....	0.05	Residue.....	0.21
Ether extract.....	0	Chloroform extract.....	0
Residue.....	0.20	Residue.....	0.21
Water extract.....	0.20		

From the experiments presented in table 3 it was possible to derive a method of extraction of a purified material (DAI) which no longer contained histamine. This material, obtained in a dry form produced a conspicuous fall of the concentration of histamine in rabbit blood (table 3, rabbit 9) and a sharp fall of carotid blood pressure in dogs, as shown in the preceding paper (1). For the experiment presented in tables 3 and 4 this DAI material was prepared from a pooled sample of the hydatid fluids, according to the following procedure: 500 cc. of the fluid was concentrated to 100 cc. under reduced pressure, and 5 grams of trichloroacetic acid was added; after standing for twenty-four hours in the icebox, the material was filtered clear, by addition of a small amount of Super-cell; the filtrate was transferred to cellophane bags and the dialysis was continued for forty-eight hours against distilled water and repeated changings of the external water; the dialyzed material was concentrated in vacuo and the syrupy residue was dried through successive additions of acetone; the dry material was collected in Buchner funnels and the acetone removed in an oven at 60°C.; the whole material



recovered amounted to 324 mgm. and was finally dissolved in 25 cc. of saline solution (solution of DAI).

The decrease of the histamine content of rabbit blood would suggest either that histamine was liberated from cells to plasma with the consequent diffusion of this substance to tissues or that a drastic reduction of blood cells produced a diminution of the histamine extractable from total blood, since there is considerable evidence that histamine is mostly bound to the leukocyte-platelet portion

TABLE 3

*Effect of the intravenous injection of hydatid fluid or products derived from it on the blood histamine of rabbits*

RABBIT	MATERIAL INJECTED*	BLOOD HISTAMINE, MICROGRAMS PER CUBIC CENTIMETER	
		Before	After
1	2 cc. (= 40 cc.) of concentrated HL	2.05	0.30
2	5 cc. (= 30 cc.) of dialysate (20 hours) of HL	3.15	3.15
3	5 cc. (= 30 cc.) of dialysate (48 hours) of HL	1.00	1.20
	5 cc. (= 30 cc.) of dialyzed (48 hours) HL	1.20	0.33
4	5 cc. (= 30 cc.) of dialysate (48 hours) of HL	1.42	1.42
	5 cc. (= 30 cc.) of dialyzed (48 hours) HL	1.42	0.40
5	5 cc. (= 100 cc.) alcohol extract of dry residue of HL	1.87	1.80
	5 cc. (= 100 cc.) of residue of alcohol extraction	1.80	0.36
6	4 cc. (= 100 cc.) of total proteins washed and dialyzed	1.46	0.83
7	4 cc. (= 100 cc.) of deproteinized and dialyzed HL	1.10	0.33
8	5 cc. (= 50 cc.) of deproteinized and dialyzed HL	2.70	0.70
9	5 cc. (= 60 mgm.) of DAI solution	1.55	0.36

\* Dialysis was performed in cellophane bags against 1 or 2 liters of distilled water. After dialysis, the external water was concentrated under reduced pressure and the final volume of the dialysate was referred to the original volume of hydatid fluid. The amounts in parentheses refer to the initial volume of hydatid fluid. The latter was concentrated under reduced pressure at 70° to 80°C. and the volume which was injected in each experiment (2 to 5 cc.) is indicated in the table. Rabbits 3, 4 and 5 were used twice, after a lag period of thirty minutes. Usually, the material which was expected to produce a decrease in the blood histamine was injected after this resting period. For the alcoholic extraction, the hydatid fluid was dried and the residue thoroughly treated with 3 to 4 portions of hot absolute alcohol. The combined extracts were dried again and dissolved in saline solution. The alcohol-insoluble residue was dried in an air current and redissolved in saline solution. The proteins were precipitated with 10 per cent trichloroacetic acid, washed with 5 per cent solution of the acid, redissolved in water and dialyzed for twenty-four hours.

of the blood. This last alternative includes two possibilities: 1, that the hydatid fluid produces a reduction of the number of leukocytes (leukopenia), or 2, that the injection of hydatid fluid into rabbits leads to a reduction of the number of circulating platelets (thrombocytopenia). These possibilities have been submitted to careful experimentation. Samples of oxalated rabbit blood were put in vitro into contact with solutions of the active material DAI which had been prepared from hydatid fluid by the procedure described previously. In a few ex-

periments, the hydatid fluid in nature was also tried, despite the fact that it contained a small amount of histamine (0.35 microgram per cc.), which has been subtracted from the final values obtained for plasma histamine. The results obtained in the in vitro experiments are described in table 4.

The in vitro experiments presented in table 4 definitely show that hydatid fluid or the active substance derived from it (DAI) is unable to liberate histamine from cells to plasma. The decrease in the blood histamine cannot therefore be explained through a mobilization of the histamine previously bound to the blood cells. The possibility that the DAI substance or the hydatid fluid itself might destroy the histamine eventually liberated from the cells was ruled out by a simple experiment. As is known, when blood clots, histamine appears in higher amounts in the plasma. In a typical experiment two samples of oxalated blood were clotted by addition of a tenth-molar solution of calcium chloride; after coagulation, an appropriate volume of DAI solution was added to one sample and the same volume of saline solution to the control sample. After centrifuga-

TABLE 4  
*Effect of hydatid fluid on the histamine content of rabbit plasma*

RABBIT	HISTAMINE CONTENT OF THE PLASMA, MICROGRAM PER CUBIC CENTIMETER		HISTAMINE IN TOTAL BLOOD, MICROGRAMS PER CUBIC CENTIMETER
	4 cc. blood + 1 cc. saline	4 cc. blood + 1 cc. DAI or HL	
1	0.20	0.20	1.5
2	0.20	0.12	1.5
3	Traces	Traces	2.5
4	0.19	0.17	1.5
5	0.33	0.28	
6	0.30	0.25	1.7
7	0.48	0.35	2.7

tion, 1 cc. of serum of each sample was extracted by Code's method and the histamine was estimated on the guinea pig ileum. Both samples contained 1.05 micrograms of histamine per cubic centimeter of serum, a fact which shows that the amount of histamine that was released in the clotting process was not altered by contact with the material derived from hydatid fluid.

The hypothesis that hydatid fluid produces changes in the number of leukocytes and platelets was verified in a large number of experiments. Hydatid fluid was injected into fourteen rabbits and the number of leukocytes was determined before and after the injection. The interesting fact that we have verified is that the reduction of the number of leukocytes depends on the conditions under which the experiments were done. Four of the five nonanesthetized rabbits and one rabbit anesthetized with dial and ether showed considerable decreases of the leukocyte count, while most of the animals anesthetized with urethane showed an increase of the circulating leukocytes (table 5). This unexpected effect of anesthesia by urethane remains unexplained but it permitted us to rule out the leukocytes as the main carriers of histamine in rabbit blood, since in almost all animals

a considerable decrease of the blood histamine followed the injection of hydatid fluid or products derived from it, as shown in table 5.

In several instances the platelets were also determined. There appeared to be almost always a decrease of the number of platelets consistent with the histamine decrease, but even here we cannot ascribe to a fall of platelets all the reduction of the blood histamine of rabbits, since in some cases the reduction of the platelet count was much more conspicuous than that of the histamine content. Although this might be accounted for by the inherent uncertainties of the platelet counting method used, the only statement one could safely make is that

TABLE 5

*Effect of hydatid fluid on blood histamine and on leukocyte and platelet counts in the rabbit*

RABBIT	ANESTHETIC AGENT USED	MATERIAL INJECTED*	HISTAMINE CONTENT, MICROGRAMS PER CUBIC CENTIMETER		LEUKOCYTES × 10 <sup>3</sup>		PLATELETS × 10 <sup>3</sup>		SHOCK PRODUCING EFFECT
			Before	After	Before	After	Before	After	
1	None	Dialyzed deproteinized HL	2.4	1.3	13.4	6.4	640	12	
2	None	Dialyzed deproteinized HL	2.7	0.7	10.4	6.4	562	62	
3	None	Dialyzed HL <sub>1</sub>	2.0	2.0	13.0	9.6			
4	None	Dialyzed HL <sub>1</sub>	0.4	0.3	10.0	12.4	480	420	
5	None	Concentrated dialyzed HL	1.4	0.4	12.2	2.8	284	66	
6	Dial and ether	Dialyzed HL <sub>1</sub>	1.1	0.9	16.8	7.4	430	397	No shock
7	Urethane	Dialyzed deproteinized HL	2.3	0.6	9.9	10.0			No shock
8	Urethane	Dialyzed deproteinized HL	2.1	0.4	14.6	16.0			No shock
9	Urethane	HL <sub>1</sub>	2.5	1.7	14.8	18.4			No shock
10	Urethane	HL <sub>1</sub>	0.5	0.5	15.6	39.7	757	239	No shock
11	Urethane	Concentrated HL	1.5	1.1	9.0	30.6	150	46	No shock
12	Urethane	Concentrated HL	3.1	2.0	16.0	20.6	108	111	No shock
13	Urethane	Concentrated HL	1.5	0.5	26.0	25.4	725	305	Moderate
14	Urethane	Concentrated HL	1.7	0.7		13.0	274	31	No shock

\* The materials were injected intravenously in amounts corresponding to 50 to 60 cc. of hydatid fluid, concentrated to 5 to 10 cc.

the histamine content of rabbit blood is more likely to depend on the number of platelets rather than on the number of leukocytes, since drastic reductions of the histamine content may coexist with no change or even an increase of the number of leukocytes in the circulating blood.

All the experiments performed on anesthetized rabbits were done with the aim of verifying whether hydatid fluid has any shock-producing effect on rabbits. In no case was there any drop of the carotid blood pressure after the injection of dialyzed or deproteinized hydatid fluid. In one case (table 5, rabbit 13), in which a concentrated, not dialyzed, hydatid fluid was injected, there was some

fall of carotid blood pressure, but this might be ascribed to some easily diffusing substance contained in the fluid. The interesting fact largely verified in the experiments described is that a sharp reduction of the concentration of histamine in rabbit blood can coexist with a lack of change in the carotid blood pressure. This fact might have some significance for the general discussion of the mechanism of anaphylactic shock in this species, presented subsequently.

COMMENT. During anaphylactic shock in the rabbit there is a marked decrease of total blood histamine, as shown first by Rose and Weil (2) and confirmed by Dragstedt and his collaborators (8), Eichbaum and Rocha e Silva (9) and many other investigators. This decrease of the total blood histamine was interpreted as resulting from the leukopenia that accompanies shock and a shift of histamine from blood cells to plasma, following combination of the antigen with the antibody (10). Trypsin, when injected intravenously into rabbits, produces the main features of anaphylactic shock: fall of blood pressure, increase of the pressure of the pulmonary artery, decrease of the histamine content of the blood, leukopenia and incoagulability of the blood, as shown by Rocha e Silva (11), Dragstedt and Rocha e Silva (3) and Rocha e Silva and Dragstedt (12). Trypsin added in vitro to samples of rabbit blood produces a release of histamine from cells to plasma, as shown by Dragstedt and Rocha e Silva (3). Also peptone, which in many respects reproduces the symptoms of anaphylaxis, produces a sharp fall of blood histamine and leukopenia in the rabbit, as shown by Gotzl and Dragstedt (4). Anaphylactic, trypsin and peptone shocks in the rabbit are therefore characterized by a triad of symptoms that have been considered by previous workers as intimately correlated: 1, fall of carotid blood pressure; 2, release of histamine from blood cells to plasma, and 3, decrease of the total blood histamine and leukopenia.

In the previous reports there was a general belief that the decrease of blood histamine should be of significance in explaining the fall of carotid blood pressure, although there is no perfect relationship between the two phenomena, as pointed out by Rose (13) and Eichbaum and Rocha e Silva (9). The experiments presented in this report show that a sharp fall of the blood histamine can coexist with a remarkably stable carotid blood pressure (no shock). The lack of parallelism between the decrease of total histamine and circulatory shock in the rabbit has been proved in the experiments presented in this paper. It is interesting to note that hydatid fluid is not able to liberate histamine from rabbit blood cells. This fact might well explain the absence of shock in rabbits given hydatid fluid intravenously, since the antigen (in sensitized animals), trypsin and peptone, which are shock-producing substances, produce a conspicuous release of histamine from cells to plasma in samples of rabbit blood. In this difference might reside the capacity or the incapacity of the foregoing substances to produce a fall of the carotid blood pressure in the rabbit.

Another point deserving discussion is the alleged relationship between rabbit blood histamine and the leukocyte-platelet count in the circulating blood.

The experiments performed with hydatid fluid introduce a new body of evidence against the view sponsored by Code (14) that histamine in rabbit blood is

mainly confined to the granulocytic series of blood elements. There has been considerable doubt as to the validity of this view. Zon, Ceder and Crigler (15) have shown that streptococcal vaccine given intravenously to rabbits produces a drop in leukocytes that cannot be correlated with the variations of the histamine content. The injection of antiplatelet serum produced the disappearance of circulating platelets and drastic reductions of the histamine content, although the leukocyte counts did not alter very significantly. Independently, Minard (16) presented evidence that 97 to 98 per cent of the histamine in rabbit blood is in the platelets. This view is undoubtedly more in accordance with the fact that, when blood clots, histamine appears in large amounts in the serum. This fact, first stressed by Code (14) was confirmed by us, using oxalated blood to which an appropriate amount of calcium chloride was added. Since platelets are the elements most concerned in the clotting process, this fact seems to afford a new argument for the idea that platelets are the most important carriers of histamine in rabbit blood.

The extensive studies of Rose (13) on anaphylaxis in the rabbit also pointed to the conclusion that the blood histamine content in the rabbit is independent of the leukocyte count. After recovery from shock the leukocytes returned to normal while the blood histamine still was at a very low level. In this case, there was, however, the possibility that the histamine previously contained in the leukocytes might be released following combination of the antigen with the respective antibody and leave the circulating blood. Direct evidences that antiplatelet serum does not release histamine are lacking in Zon, Ceder and Crigler's paper (15). Hydatid fluid has no ability to release histamine, as shown in the foregoing experiments, and yet it produces a sharp fall of blood histamine. Since in several cases there was no corresponding decrease of circulating leukocytes, this definitely shows that the leukocytes are not important carriers of histamine in rabbit blood although we cannot be sure that the platelets are the only carriers of this toxic substance. At present we can only assume that proofs are more in favor of the platelets than of the leukocytes as being the carriers of histamine in rabbit blood.

#### CONCLUSIONS

Hydatid fluid contains histamine in both free and bound conditions. The conclusion is drawn that histamine is bound, forming a low molecular complex that is fairly soluble in alcohol but insoluble in ether, acetone or chloroform. This inactive histamine "precursor" yields free active histamine after prolonged boiling in the presence of concentrated hydrochloric acid.

When injected intravenously, hydatid fluid produces a drop of the blood histamine and very mild symptoms in the rabbit. This ability of hydatid fluid to produce a reduction of the total blood histamine is not impaired by dialysis against distilled water, nor by deproteinization by trichloroacetic acid. From pooled samples of hydatid fluid a purified material was prepared, which has the same capacity of reducing the total blood histamine when injected intravenously into rabbits. In vitro experiments have shown that hydatid fluid and the puri-

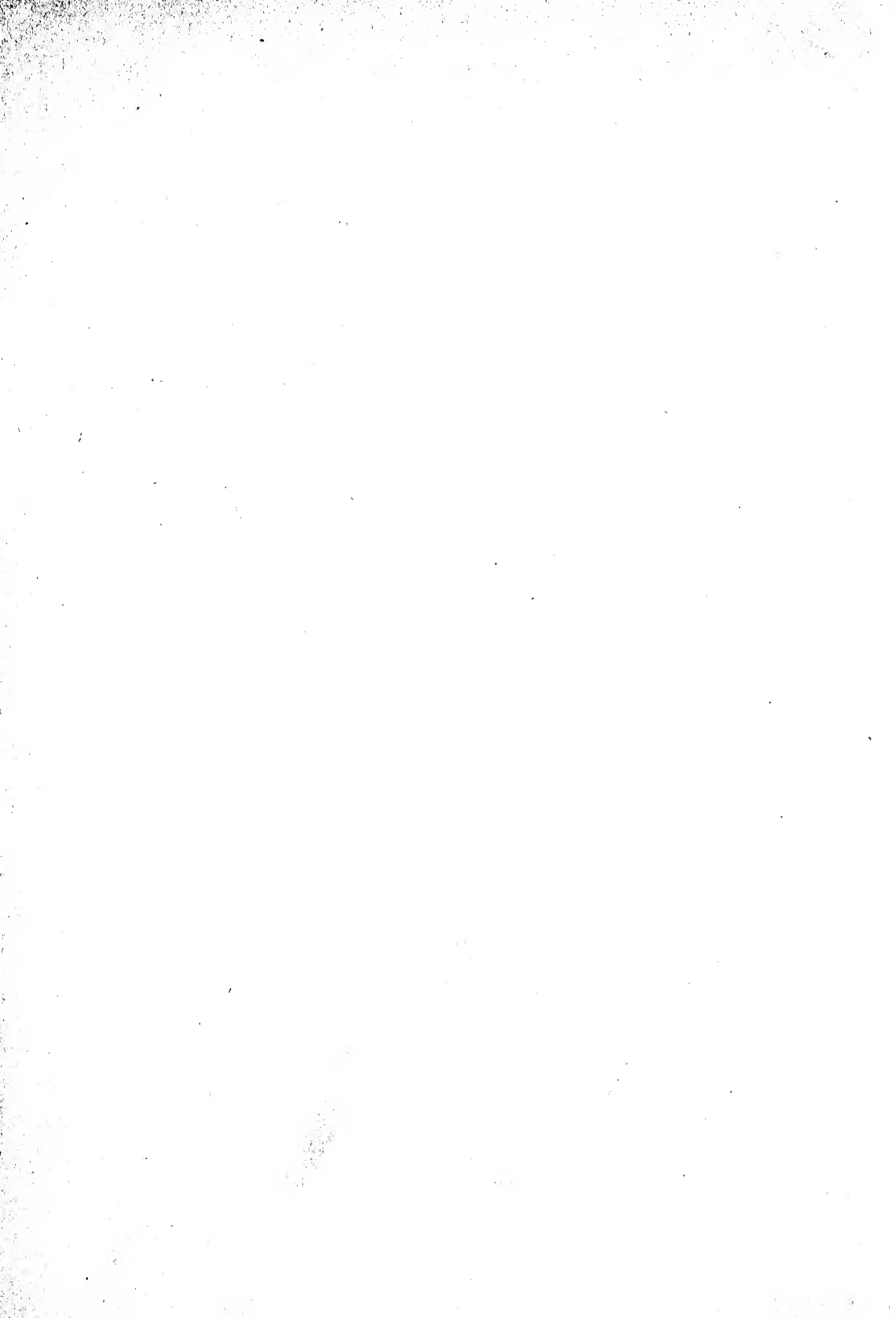
fied material derived from it are not able to liberate histamine from cells to plasma in samples of rabbit blood.

In nonanesthetized rabbits the intravenous injection of hydatid fluid results in leukopenia and thrombocytopenia, while in rabbits anesthetized with urethane there was leukocytosis accompanied by a drop in the platelet count. In all cases, however, there was a sharp reduction of the histamine content of the blood. From these experiments, the conclusion is drawn that histamine is more likely to be carried by platelets than by leukocytes, in the circulating blood of the rabbit.

Hydatid fluid, or products derived from it, do not produce a fall of carotid blood pressure in the rabbit under dial or urethane anesthesia, even when a sharp reduction of the blood histamine is observed. From this fact the conclusion was drawn that there is independence between a decrease in blood histamine and the occurrence of shock.

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## THE NERVOUS MECHANISMS OF THE MUSCULARIS MUCOSAE<sup>1</sup>

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The movements of the intestinal villi in living animals were first described by Gruby and Delafond (1). Since the appearance of their report in 1843, contributions to this subject have appeared sporadically. Among those dealing to a large extent with the movements of the villi and giving little or no consideration to the activities of larger masses of the muscularis mucosae are contributions by Bruecke (2), Hambleton (3), King and Arnold (4), Verzář and MacDougall (5), Kokas (6), Ludany and Jourdan (7), and Wells and Johnson (8). The subject matter in these treatises centers largely around descriptions of the movements of the villi, the nature of the stimulus or stimuli, and an attempt to correlate the activity of the villi with the processes of secretion and absorption.

In a smaller group of reports primary attention has been given to the activities of larger masses of the muscularis mucosae. Papers by Exner (9), Gunn and Underhill (10), King and Church (11), Thorell (12), and Forssell (13), have dealt with various aspects of this subject.

There is a fairly close agreement among the different writers in their descriptions of the types of contractions executed by the muscularis mucosae. The more fundamental problems, however, dealing with the nature of the normal physiological stimulus or stimuli, the myogenic or neurogenic origin of the contractions, the physiological function of the contractions, and the rôle of the nervous mechanisms present have remained unsettled.

With respect to innervation there is general agreement that the sympathetic system supplies excitatory fibres to the muscularis mucosae of the small intestine, rather than inhibitory as in the case of the outer musculature. The function of the parasympathetics has not been so well worked out. Thorell (12) found that as a general rule the muscularis mucosae of the stomach was caused to contract by acetylcholine although in some animals this reaction was obtained only from certain regions of this organ. King and Church (11) noted the contractile effect of pilocarpine on the muscularis mucosae of the dog's small intestine.

<sup>1</sup> The expenses of this investigation were defrayed by a grant from the Bristol-Myers Co.



tine but did not study the effects of acetylcholine. The conventional view is that the sympathetic supply to the gastro-intestinal tract is entirely post-ganglionic and that all the units of the intrinsic plexuses are parasympathetic in origin. A few neuro-histologists (14) contend that both parasympathetic and sympathetic cells are present in the plexuses of Meissner and Auerbach.

The results herewith reported prove that the muscularis mucosae of the large and small intestine of the dog is innervated by both cholinergic and adrenergic excitatory nerves, and supply evidence indicating that ganglion cells with cholinergic terminations and cells with adrenergic endings are both present in Meissner's plexus.

The experimental procedures may be divided into two series: first, in dogs under barbital-sodium anesthesia, areas of the intestinal mucosa were observed with the aid of a binocular microscope during the stimulation of parasympathetic and sympathetic extrinsic nerves, and also during and following the intravenous administration of various drugs; in the second series the reactions of isolated segments of intestine from which the outer musculature had been removed were observed and recorded during and following the introduction of drugs into the environmental bath.

*Observations of the movements and reactions of the intestinal mucosa in intact anesthetized dogs.* The method of preparing the mucosa for observation has been described elsewhere (4). The chief precautions involved are to reduce the interference with the circulation to a minimum, avoidance of undue stretching while fastening the edges to the mounting board, and keeping the surface moist and warm. In the present experiments the field was constantly irrigated with a saline drip warmed so that the outlet temperature was approximately 39°C. A drop of several degrees below this temperature produces immediate diminution in activity which might be confused with or mask the effects of experimental procedures. If these precautions are carefully observed one seldom fails to secure a reactive field and one which shows spontaneous activity for many hours.

Two types of spontaneous activity were manifested under these conditions. One, confined to the individual villi, consisted of swaying, shortening, and lengthening, and was often quite rhythmical. This activity was sharply localized; other villi in the neighborhood of the active one often remained quiescent, and there was no evidence of a wavelike spread of excitation. The other type of activity consisted of changes in the topography of the surface, with the appearance of grooves and ridges which often involved the whole field in view under the microscope, or even the whole of the exposed surface. It was at times difficult for the observer to decide whether these changes were incident to activity of the outer musculature or to that of the muscularis mucosae. However, the appearance and disappearance of ridges and grooves were frequently observed when there was no visible evidence of activity on the part of the outer circular and longitudinal muscles.

A few observations were made on the reaction of the mucosa to sharply localized mechanical stimulation. On touching the tip of a villus with a finely drawn out glass rod we have observed only occasional contraction. More

vigorous stimulation, producing distortion of the upper half of the villus, was more often effective. When the tip of the rod was pushed into the mucosa at the base of the villus a contraction practically always occurred which involved not only the villus stimulated but also a group of adjacent villi. This reaction was always accompanied by the formation of a crater-like depression in the mucosa centered around the locus of stimulation. This region soon regained its normal appearance after the withdrawal of the stimulus.

Four animals were used for the purpose of studying the effects upon the mucosa of stimulating the extrinsic nerves, vagi and splanchnics. Our findings corresponded closely with those previously reported from this laboratory (4) but are at variance with some of the results presented by Ludany and Jourdan (7). These authors reported observing a fleeting initial contraction incident to peripheral vagal stimulation, followed by complete cessation of activity which lasted throughout the duration of stimulation.

We have not observed complete cessation of the activity of the villi during peripheral vagal stimulation in any of our experiments. In some instances a slight diminution in activity was noted, in others a slight increase, but usually no change. Ridging of the mucosa, however, commonly occurred. These tests were made with stimuli ranging in intensity from that just sufficient to produce slight cardiac slowing to that producing complete cardiac arrest. Atropine sulphate was injected in a few experiments in doses just sufficient to prevent cardiac slowing. It is well known that doses of this magnitude are not large enough to prevent a response of the outer musculature of the gut incident to peripheral vagal stimulation. The presence of the drug did not materially alter the responses of the mucosa.

Stimulation of the splanchnic nerves always resulted in marked pallor of the mucosa, but the initial effect upon the contractions of the villi and the topography of the mucosal surface was variable. Sometimes the villi immediately retracted sharply and ridges and grooves appeared, at other times the mucosa remained quiescent for some seconds. However, later, regardless of the nature of the initial changes, there was always an increase in the activity of the villi both as to the number contracting and in the rate of their contractions. This augmentation of activity passed off within several minutes. It was noted that the onset of the increase in their activity coincided closely with the secondary rise in the arterial blood pressure.

The mucosa was also observed during and following the intravenous injection of 5 cc. of 1:100,000 solution of acetylcholine. This was sufficient to cause a marked slowing of the heart, considerable fall in arterial pressure, and vigorous activity of the outer musculature of the small intestine. The changes in the mucosa corresponded qualitatively to those observed during and following electrical stimulation of the vagi. The ridging of the mucosa was more pronounced following the injection of the drug than following vagal stimulation but the effect on the activity of the villi was not materially different.

Following the intravenous injection of epinephrine in doses of 5 cc. of 1:100,000 solution the mucosa quickly became very pale, the villi contracted and the

whole surface became ridged. During the time the mucosa was pale and the villi retracted there was no rhythmical movement, but at a time corresponding closely to the peak in the rise in arterial pressure the surface became pink and the villi began to move rhythmically. In most instances the movements gradually increased, often became more pronounced than before the drug had been administered, then gradually returned to their original state of activity.

In one animal 1.0 mgm. of nicotine was injected intravenously while the intestinal mucosal surface was being observed. The mucosa became very pale, the villi contracted sharply and marked pitting, grooving and ridging appeared. Qualitatively the reaction of the mucosa was similar to that observed in the same animal following the injection of epinephrine but was more intense. Nicotine also caused vigorous contractions of the outer musculature of the gut which did not occur following epinephrine. During the time the villi were retracted they manifested no spontaneous movements but after the lapse of about a minute the surface became pink, the villi elongated and individual movements began. This occurred before the respiratory excitement and the contractions of the outer layers of intestinal muscles had subsided.

The results of these experiments show that contraction of the muscularis mucosae can be initiated by stimulation of extrinsic parasympathetic and sympathetic nerves supplying the small intestine of the dog, by drugs acting in the region of the terminations of motor nerves, and by drugs acting on ganglion cells in the pathway of innervation. For the purpose of further clarifying the nature of the peripheral (intrinsic) nervous mechanism involved, experiments on surviving isolated segments of the muscularis mucosae were done.

*Experiments with surviving segments of muscularis mucosae.* The term muscularis mucosae is here used to designate preparations from the gastro-intestinal tract from which the outer circular and longitudinal muscular layers have been removed.

In all experiments in which fresh material was used the animal was kept alive, under anesthesia, and the segments removed as needed. The routine procedure was to remove the part and immediately submerge it in saline solution at 5°C. where it was left undisturbed for from 15 to 30 minutes. The solution used was made up by adding salts to distilled water in the proportions used by Tyrode with the exception that no magnesium was added. In this paper this solution is hereafter designated as saline solution. The removal of the outer muscle layers proved to be more easily accomplished and with less damage after a preliminary period of chilling. Segments which were to be used several hours or even days after removal were first flushed out with cold saline solution, then immersed in fresh solution and stored in a refrigerator maintained at a temperature of 4 to 6°C. A few segments were removed several hours after the death of the animal. They retained a fair degree of viability but a longer time was required to establish a steady state and they definitely deteriorated more rapidly than fresh material treated as described above.

The segments, about 4 cm. long, were mounted in a chamber containing 200 cc. of saline solution, and the chamber was partially submerged in a water bath

maintained close to 39°C. The oxygen supply was obtained by means of a constant stream of filtered room air. The attachments to the recording lever and to the holder were made at opposite ends of the segment and placed diagonally to each other. We have not found a satisfactory method for registering contractions of circular muscle, if such exists in the muscularis mucosae. A slightly modified Trendelenberg method gave records of longitudinal contractions but no conclusive evidence of circular activity. For recording, a light well balanced lever of the first class, giving a magnification of 18 times was used.

When first placed into the warm saline solution, the segments gradually lengthened until after the lapse of from 5 to 10 minutes they assumed a fairly constant length. As a rule rhythmical movements did not begin until the length had approached a constant level. In many instances the saline solution was at room temperature when put into the chamber and the tissue was immersed when the temperature was considerably below 39°C. Rhythmical contractions seldom began before the bath had warmed to 35°C. or even higher, although this may have been partly a diffusion rather than solely a temperature effect. As a general procedure drugs were not added to the environmental fluid until a constant activity of the tissue had been established.

1. *The effects of epinephrine.* Preparations from all levels of the small intestine and from the middle portion of the large intestine responded with a sustained shortening following the addition of epinephrine to the bath (fig. 1-A). Rhythmical contractions, if present before the drug was added, usually persisted, and more often than not at a slightly accelerated rate throughout the duration of the response. No primary relaxation was noted in any of the tests in which epinephrine was the only drug added. The minimum dosage required varied considerably with different preparations. Some reacted to dilutions of 1:200,000,000. There were no failures to contract with fresh tissues when the concentration was 1:20,000,000, and concentrations of from 1:2,000,000 to 1:1,000,000 usually sufficed to induce a maximal reaction to the drug. Previous treatment with atropine or nicotine did not prevent the contractile response to epinephrine, but ergotamine tartrate in concentrations of from 1:100,000 to 1:120,000 proved to be an effective antagonist. Tissues, if prepared as previously described and then refrigerated, were only slightly less reactive after 24 hours than when freshly removed from the body, but after 96 hours the majority reacted slightly or not at all regardless of the amount of drug added. One specimen gave a small reaction to drugs after it had been refrigerated for 10 days. In no instance, if the aged tissue failed to react to epinephrine, were we able to induce contraction by means of histamine or barium. This statement also applies to aged preparations which failed to be affected by acetylcholine.

2. *Effects of acetylcholine bromide.* The reaction to acetylcholine invariably consisted in the shortening of the segment (fig. 1-B). As in the case with epinephrine, there was considerable variation between different fresh preparations in the minimal concentration of the drug necessary to elicit a response. A few reacted to 1:200,000,000, the majority to 1:20,000,000, and in most instances 1:2,000,000 sufficed to induce a maximal contraction. The effectiveness of

acetylcholine was not impaired by previous nicotization or by the addition of sufficient ergotamine tartrate to abolish the effect of epinephrine. Atropine prevented the reaction to acetylcholine, but as stated previously did not affect the response to epinephrine (fig. 2). As a rule the maximal shortening induced by

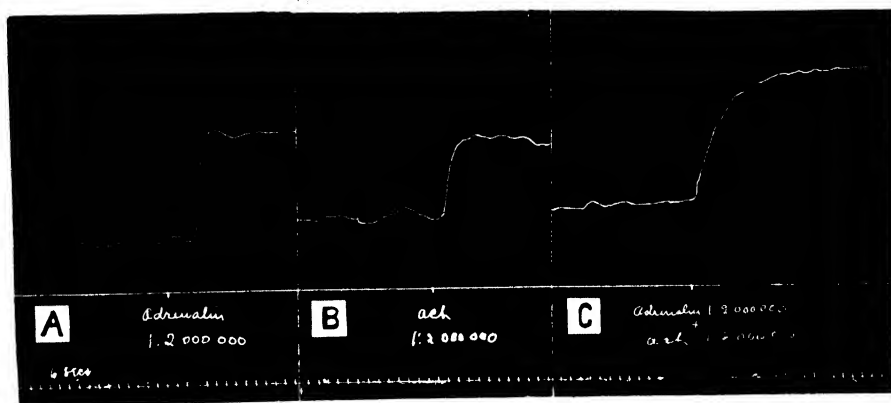


Fig. 1. Reactions of the same segment of muscularis mucosae from the upper jejunum, fresh tissue. A, the reaction to 1:2,000,000 adrenalin; B, the reaction to 1:2,000,000 acetylcholine; C, the reaction to 1:2,000,000 adrenalin and 1:2,000,000 acetylcholine introduced simultaneously. Time intervals, 6 seconds. Simultaneous ordinates not shown, but they were constant for the three records.

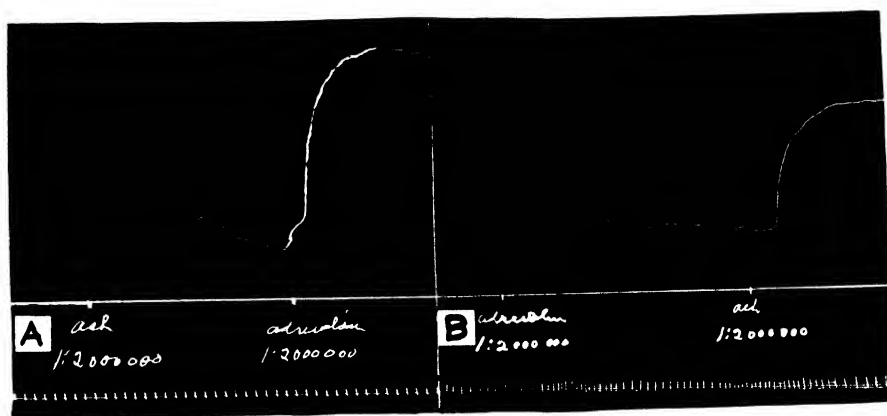


Fig. 2. Reactions of separate fresh segments of muscularis mucosae from adjacent levels of the upper jejunum. A, a segment previously atropinized showing the absence of reaction to acetylcholine and the reaction to adrenalin; B, a segment previously ergotaminized showing the failure to react to adrenalin and the reaction to acetylcholine. Time intervals, 6 seconds.

acetylcholine was not as great as that following the introduction of epinephrine (fig. 1-B).

With but a few exceptions the time elapsing between the introduction of the drugs into the bath and the beginning of the contraction was considerably

shorter for acetylcholine than for epinephrine (figs. 1-A, 1-B). While the technique for introducing the drugs and the method of signaling were not precise enough to determine latency very accurately, this procedure and also the mixing and dispersion of the drugs in the bath was fairly uniform, so that it is not likely that the difference noted was fortuitous. The significance of this difference must for the present remain conjectural. The structures affected by acetylcholine may be more superficial, the drug may penetrate tissues more rapidly than epinephrine, or there may be a fundamental difference between the structures affected.

3. *Acetylcholine and epinephrine added to the bath simultaneously.* The height of the contraction induced when epinephrine and acetylcholine were put into the environmental fluid at the same time, while higher than the contractions elicited by each drug alone was never their sum (fig. 1-C). The deficit in the height of the contraction when both drugs were used simultaneously cannot be

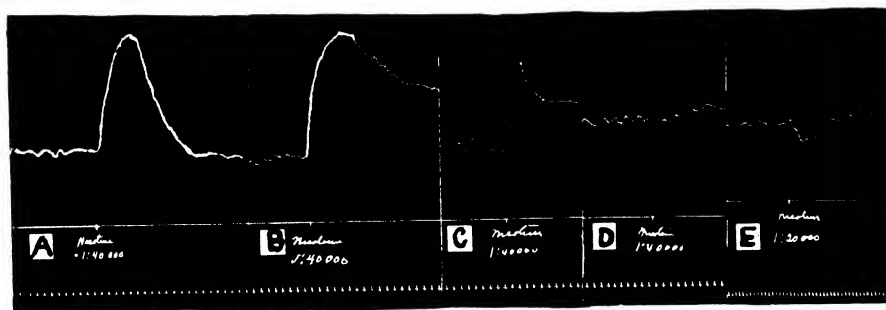


Fig. 3. Typical reactions of the muscularis mucosae of the jejunum to nicotine under different conditions. A, fresh segment; B, fresh segment from the same animal after atropinization; C, segment after treatment with ergotamine; D, segment after previous additions of atropine and ergotamine; in a subsequent test this preparation showed a slight contractile reaction to adrenalin. E, a segment which had been refrigerated for 38 hours previous to the test. Time intervals under E, 3 seconds, under others, 6 seconds.

accounted for on the basis of deterioration of the tissue, because in almost every instance the reactions to each drug in control tests following the test with both used concurrently, were of the same magnitude as in preliminary control tests.

4. *Effects of nicotine.* Nicotine was employed in isolated preparations of the muscularis mucosae as a test for functional ganglion cells and also as a part of the procedure for their differentiation.

Preparations of fresh isolated segments of muscularis mucosae invariably contracted following the initial dose of nicotine (fig. 3-A). This effect occurred after atropinization sufficient to prevent a response to acetylcholine (fig. 3-B), and also after treatment with ergotamine tartrate in an amount adequate to prevent a reaction to epinephrine (fig. 3-C). After treatment with both atropine and ergotamine, however, nicotine failed to induce any marked contraction (fig. 3-D) instead, a small brief relaxation usually occurred.

The effect of the initial dose of nicotine on preparations which had been re-

frigerated for several days or longer differed from that found with fresh material. The usual reaction was biphasic, either a slight contraction followed by relaxation, or a relaxation followed by a slight contraction (fig. 3-E, preparation 38 hrs. old). These effects were of short duration and the segments soon assumed their original lengths. This type of response was encountered only twice with tissues which had been in the refrigerator less than 24 hours, and in both instances they had been removed from the animal several hours after death.

The dosages of nicotine employed, ranging from 1:40,000 to 1:20,000, were sufficient to establish a state in the tissues such that succeeding doses were without effect. Under these conditions spontaneous rhythmical movements were not abolished, nor was the reactivity to acetylcholine or epinephrine appreciably altered.

5. *Segments from the large intestine.* Only two experiments were done on material prepared from the large intestine. The removal of the outer musculature from the large intestine was more difficult than from the small intestine, chiefly because of the greater abundance of connective tissue strands extending from the outer into the inner layers. The spontaneous activity of these segments, their reactions to drugs and the duration of their survival were qualitatively similar to the results obtained with material from the small intestine. Histological studies of the intrinsic plexuses of the large intestine have not been as extensive as those made on the plexuses of the small intestine, but to our knowledge no fundamental structural difference between them has been demonstrated. The results presented in this report support this view from a functional standpoint.

DISCUSSION. 1. *Assumptions with regard to drugs used.* The validity of any physiological conclusions drawn on the basis of the reaction of animals and of tissues to drugs depends upon the correctness of the assumptions with reference to the loci and nature of the actions of the drugs.

Acetylcholine is assumed to produce its effect, when injected into intact animals, by its action in the region of nerve endings in peripheral tissues, by its effects on ganglia in the chain of innervation, and also possibly by its central effect. Hence the observation that the injection of acetylcholine into dogs is followed by a definite change in the topography of the intestinal mucosa does not in itself prove the presence of nerves with cholinergic endings in the muscularis mucosae. In like manner, the contraction produced by acetylcholine in isolated segments of the muscularis mucosae might be due to the stimulating action of the drug on ganglion cells of Meissner's plexus with excitatory adrenergic terminations. (Segments of muscularis mucosae prepared as described are devoid, or nearly so, of cell bodies of Auerbach's plexus, but Meissner's plexus is left intact.)

It is assumed that epinephrine acts peripherally at the effector cells in the region of the termination of adrenergic nerves.

With respect to nicotine, the conventional view is adopted that the initial reactions to the drug are due to its stimulating effect on ganglion cells; an effect which is soon replaced, if the dose is large enough, by depression or paralysis of the nerve cells.

2. *Evidence for two kinds of excitatory motor ganglion cells in Meissner's plexus.* Since it is well known from histological studies that Meissner's plexus is rich in ganglion cells, the initial contraction produced by nicotine in segments of fresh muscularis mucosae is interpreted as being due to the drug's stimulating effect on the ganglia, and the failure to obtain contractions following subsequent doses of nicotine is considered evidence that the paralytic stage has supervened.

That the nicotine is acting only on the ganglia in these experiments is further supported by the following facts: first, typical contractile reactions are induced by nicotine in fresh segments of the muscularis mucosae after previous treatment with either atropine or ergotamine alone, but do not occur if both atropine and ergotamine have previously been added; secondly, the induction of the paralytic state by nicotine does not alter the character of the reactions to either epinephrine or acetylcholine, neither is the tone of the segment (length) or the character of its spontaneous movements appreciably affected.

In our opinion, therefore, these facts are best interpreted as indicating the presence in Meissner's plexus of motor nerve cells whose terminations are cholinergic and of functionally similar cells whose endings are adrenergic. The presence of motor endings extrinsic in origin, of either or both types, is not excluded.

The data at hand do not permit the formation of definite conclusions with reference to the proportions or distribution of different motor elements in Meissner's plexus. In the intact dog the more pronounced effects of splanchnic stimulation and of epinephrine on the activity of the villi as compared with the slight and indefinite effects of vagal stimulation and of acetylcholine, might be interpreted as indicating that the muscular layer from which come the strands extending into the villi is predominantly innervated by nerves with adrenergic endings. This problem still awaits further investigation. The more pronounced reaction of the villi and the more marked ridging and grooving following the injection of nicotine into the intact animal is interpreted to be due to the simultaneous stimulation of all the motor cells in the submucous plexus.

3. *The question of intrinsic inhibitory nerves in the muscularis mucosae.* The evidence available to us for the existence of an inhibitory mechanism in the muscularis mucosae is scanty and inconclusive. We have not been able to confirm the observations of Ludany and Jourdan (7) that peripheral vagal stimulation causes any inhibition of the activity of the villi. The picture as seen by us both during vagal stimulation and following the injection of acetylcholine, conforms better to what one might expect from a contraction of a considerable portion of the muscularis mucosae with little or no effect upon the musculature of the villi. Stimulation of the splanchnic nerves and the injection of epinephrine induce a brief state of quiescence in the villi and also in the whole mucosal surface; but rather than a relaxation, it is a quiescence associated with a ridged and grooved mucosal surface and retracted villi, a picture not characteristic of inhibition.

The relaxation induced by nicotine in isolated segments of the muscularis mucosae which have previously been treated with atropine and ergotamine might be construed as indicating the presence of ganglion cells with adrenergic inhibi-



tory endings. The relaxation of aged preparations incident to the introduction of nicotine into their environs might also be explained on the same basis. In this case it would be necessary to assume that the inhibitory ganglion cells remain viable longer than the excitatory cells. As an alternative hypothesis one might conceive the relaxing effect of nicotine under the conditions described to be due to a direct depressant effect of the drug on the muscle cells. Regardless of the true nature of this reaction, it is a relatively feeble one, and in the intact animal or in fresh untreated isolated segments it could easily be entirely overshadowed by the marked contractile reaction induced by nicotine. Further study is needed to furnish a more conclusive answer to this question.

4. *The myogenic origin of the contractions. Effects of aging.* Spontaneous movements occur in fresh material after the preparations have been rendered non-reactive by ergotamine and atropine to either epinephrine or acetylcholine, and are demonstrable in aged tissues so long as the segments will react to either barium or histamine. The view that the contractions of the muscularis mucosae are basically myogenic in origin is, therefore, supported. Our results present evidence that in the aging muscularis mucosae the mechanisms through which nicotine mediates contraction disintegrate earlier than those through which acetylcholine and epinephrine act. With reference to the latter two drugs we have obtained no evidence for any difference in the duration of reactivity to them in the aging muscularis mucosae. Furthermore, aged tissues which react only feebly or respond not at all when treated with acetylcholine or epinephrine behave in like manner when tested with histamine or barium.

5. *Functions of the muscularis mucosae.* In current textbooks of physiology the discussions relative to the physiological rôle of the muscularis mucosae deal almost entirely with the movements of the villi and their possible effects upon the processes of secretion and absorption. The most frequently expressed view is that the villus acts as a pump thus facilitating the flow of lymph from the intestine. In the present investigation no particular effort was made to clarify this aspect of the problem. In an earlier paper (4) King and collaborators, because they could obtain no conclusive evidence of a pumping effect, suggested that the movements of the villi serve to facilitate absorption because of a local stirring or agitating effect. Wells and Johnson (8) also were not able to support the "pump" theory and emphasized the importance of the circulatory changes in the villi induced by their movements.

The muscularis mucosae is well developed in the gastro-intestinal tract of mammals and birds, although its thickness is not the same for all species. There are no villi in the stomach and large intestine, and even in the small intestine that portion of the muscularis mucosae which extends into the villi represents only a minor fraction of the whole musculature. Because of these facts alone it is difficult for one to escape the conclusion that the activity of the villi, whatever this accomplishes, represents only a small part of the activity and function of the muscularis mucosae as a whole. On the basis of numerous observations of the spontaneous and induced changes in the topography of the mucosal surface in intact animals, and the recorded activities of isolated segments, the authors

are of the opinion that the activities of the muscularis mucosae can and do affect the transport and disposition of material in the gastro-intestinal canal. This view in recent years has been accorded increasing favor among roentgenologists, pioneered by Forssell (13).

While the muscularis mucosae appears to be essentially an autonomous mechanism, the nature of its innervation is such that outbursts of autonomic activity, whatever the cause, could profoundly affect its movements. The effects of great emotional stress upon the digestive tract may thus in part be due to changes in the activity of the inner musculature, rather than entirely to changes in the activity of the outer layers.

#### SUMMARY

1. The muscularis mucosae of the small and large intestine of the dog is innervated by both cholinergic and adrenergic motor nerves.
2. Meissner's plexus contains ganglion cells the endings of which are cholinergic, and also cells with adrenergic endings.
3. No conclusive evidence has been obtained for the presence of an inhibitory neural mechanism in the muscularis mucosae.
4. The rhythmic movements of the muscularis mucosae are basically myogenic, but can be initiated or augmented through its nervous mechanism.
5. The muscularis mucosae may play an important mechanical rôle not directly connected with the processes of secretion and absorption.

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# A COMPARISON OF THE NUTRITIVE VALUE OF DEXTROSE AND SUCROSE AND OF THE EFFECTS PRODUCED ON THEIR UTILIZATION BY THIAMINE HYDROCHLORIDE

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This study deals with a comparison of the nutritive values of dextrose and sucrose and of the effects produced by thiamine hydrochloride on their utilization. Most of the previous determinations of the nutritive values of these sugars were made under conditions in which each sugar constituted one of several other ingredients of the diet. In the present study the so-called "single food choice" technique was used in which one sugar constituted the entire diet (1-3).<sup>2</sup> To compare the nutritive values the following criteria were used: the length of time rats of approximately the same age and weight survived on each of the sugars; their energy as measured by spontaneous running activity; their food and water intake; the condition of their reproductive tracts as reflected in the vaginal smears. The experiments were carried out in three series. In the first the rats had access to only one sugar and water; in the second they had access also to a 0.02 per cent solution of thiamine hydrochloride; in the third they had access to cod-liver oil in addition to the thiamine solution.

The results of the single food choice studies on dextrose have already been published (4). It was shown that while rats of a standard weight survived only 4 days without any food, they survived 37 days on dextrose as the sole source of nourishment and twice as long (75 days) when given access also to a solution of thiamine hydrochloride. These experiments demonstrated under the simplest conditions the nutritive value of dextrose and the great effect produced by thiamine hydrochloride on its utilization.

The data on the nutritive value of sucrose which have recently been obtained with the same method are now compared with the previously collected data on dextrose.

**METHODS.** In these experiments the rats were kept in individual all-metal cages which consisted of a revolving drum (diameter 13 in.) with a ratchet cyclometer to record the number of revolutions, and a small living compartment with a nonspillable food-cup and two graduated bottles. The structure of the cages was such as to greatly reduce, if not completely eliminate, coprophagy which, as pointed out in a previous paper, plays an especially important part in the single food choice experiments (4).

<sup>1</sup> These experiments were carried out under a grant from the Corn Industries Foundation New York City.

<sup>2</sup> Dr. L. Emmett Holt with whom this single food choice technique was developed has subsequently focused his interest on the needs of the organism during conditions of inanition (2, 5) while we have focused our interest more on the general problem of differences in the nutritional values of various purified and natural food stuffs.

Records of activity, food and fluid intake and of vaginal smears were taken daily, and body weight was recorded weekly. The activity drums were tested bi-weekly to make certain that they all revolved with equal ease.

The temperature of the room which housed the cages was kept as nearly constant as possible. It averaged 75 degrees and ranged between 73 and 80 degrees.

The rats were placed in the activity cages and fed the stock diet at an average age of 45 days. After 15 to 20 days, when the body weights were between 120 and 149 grams, the single sugar replaced the stock diet.

Thirty-six female rats were used in the sucrose experiments: 11 rats on sucrose alone; 13 rats on sucrose and a 0.02 per cent solution of thiamine hydrochloride; 7 rats which for 10 days previous to the start of the single food diet had had access to cod-liver oil and then were given sucrose and the thiamine solution; and 5 rats which from the start of the single food diet had access to cod-liver oil in addition to the thiamine solution. For purposes of comparison we have used from our previous experiments on dextrose the records of 33 female rats with starting weights between 120 and 149 grams, 21 on dextrose alone and 12 on dextrose and the 0.02 per cent solution of thiamine hydrochloride. Observations were made also on 10 rats given dextrose which had access to cod-liver oil in addition to the thiamine, and on 12 rats which had access only to water and to no food.

**RESULTS.** Table 1 summarizes the results of the effects produced on the survival times. For 11 rats on sucrose and for the 21 rats on anhydrous dextrose the survival times averaged exactly the same, namely 37 days. For the 13 rats on sucrose and thiamine hydrochloride the survival time averaged 56 days, that is, 19 days longer than on sucrose alone, while for the 12 rats on anhydrous dextrose and thiamine hydrochloride the survival times averaged 74 days, that is 37 days longer than on dextrose alone. Thus it was shown that the thiamine hydrochloride increased the survival time of the sucrose-fed rats 51.4 per cent and that of the dextrose-fed rats 100 per cent. It is interesting that the rats in this latter group were much more consistent in their survival times, dying between the 62nd and 87th day while those on the sucrose plus vitamin diet died between the 35th and 77th day.<sup>3</sup>

*Energy or spontaneous activity.* Figure 1A shows the results of the observations made on activity. It shows the average daily running activity for the last 10-day period on the stock diet and for as many 10-day periods thereafter as the rats survived on the single food. The rats on dextrose and sucrose were almost equally active. For the last 10-day period on the stock diet the daily activity

<sup>3</sup> Using animals of 30 to 35 days of age and weighing 63 to 68 grams Holt and Kajdi (5) found that rats on sucrose lived on the average 29.3 days and on dextrose 27.3 days, and when given access to a 0.01 per cent solution of thiamine hydrochloride the rats on dextrose lived 49.8 days. No observations were made on sucrose-fed rats with access to thiamine. In our previous experiments (3) in which the rats were older than those used by Holt and Kajdi and in which coprophagy was only partially eliminated we found that on dextrose the rats survived on the average 57 days, while on sucrose they survived only 42 days. The greater effect on the dextrose rats of the thiamine contained in the feces probably accounts for this difference in survival times.

of the two groups averaged 12,150 and 15,110 revolutions respectively. For the first 10-day period on the single food diet the activity of both groups of rats increased. After that the activity decreased, slowly at first then more rapidly, finally reaching an average near 2,000 revolutions in both groups.

The same figure also shows the activity records of the two groups of rats which had access to thiamine hydrochloride. For four 10-day periods on the single food choice both groups of rats showed almost exactly the same activity

TABLE 1

DIET	NUMBER OF RATS	AVERAGE AGE AT START	AVERAGE WEIGHT AT START	SURVIVAL TIME	AVERAGE SURVIVAL TIME
		days	grams	days	days
Sucrose.....	11	63 (56-66)	133 (124-145)	29, 31, 33, 34, 34, 38, 39, 40, 42, 46	37
Dextrose.....	21	64 (56-71)	137 (120-149)	28, 29, 32, 33, 33, 34, 35, 36, 36, 36, 37, 37, 38, 39, 39, 40, 40, 41, 42, 42, 54	37
Sucrose + B <sub>1</sub> .....	13	62 (55-66)	138 (127-148)	35, 40, 45, 47, 47, 50, 55, 57, 66, 69, 70, 75, 77	56
Dextrose + B <sub>1</sub> .....	12	62 (58-66)	139 (130-148)	62, 65, 67, 72, 73, 74, 74, 75, 76, 76, 87, 87	74
Sucrose + B <sub>1</sub> (cod-liver oil 10 days before stock diet).....	7	68 (66-74)	142 (134-148)	55, 56, 70, 71, 77, 92, 96	74
Sucrose + B <sub>1</sub> + cod-liver oil.....	5	69 (68-69)	134 (123-149)	55, 57, 63, 73, 77	65
Dextrose + B <sub>1</sub> + cod-liver oil.....	10	67 (61-73)	140 (130-144)	56, 68, 70, 73, 78, 79, 88, 90, 90, 109	80
No food.....	12	60 (58-68)	133 (119-149)	3, 4, 4, 4, 4, 4, 4, 4, 4, 5, 5	4

curves. After that the rats on sucrose showed a sharp decrease in activity, while the rats on dextrose still remained fairly active for 20 days more before they showed the same sharp decrease. During the 50-60 day period the rats on dextrose still ran more than 5 miles per day.

*Body weight.* Figure 1B gives the average body weights for the four groups of rats. For the first 20 days all four curves closely paralleled one another. After that both groups of rats on the sugars without the vitamin supplement lost weight at a more rapid rate than did those which had access to the thiamine

hydrochloride solution. In both instances, however, the rats of comparable groups on dextrose and sucrose lost weight at essentially the same rate.

**Food intake.** Figure 2A gives the average daily intake of dextrose and sucrose for the rats without and with access to thiamine hydrochloride. During the 10-day control period on the stock diet both groups of sucrose-fed rats ate slightly less food than did the dextrose-fed rats, and when placed on the single food diet they continued to eat consistently about 15 per cent less than did the dextrose-fed rats.<sup>4</sup> In order to determine whether this was a real or only an apparent difference the food intake was calculated in calories per kilogram body weight. Figure 2B shows these curves. On the sugar alone the rats ate def-

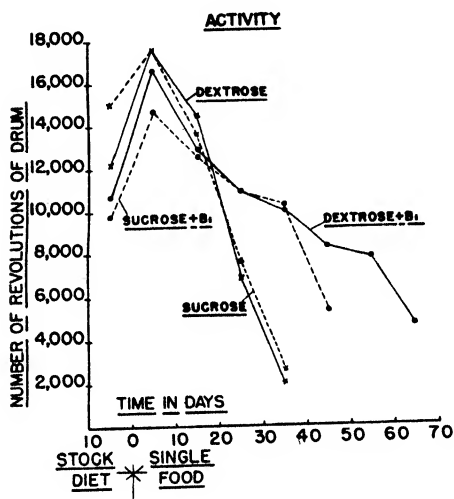


Fig. 1A

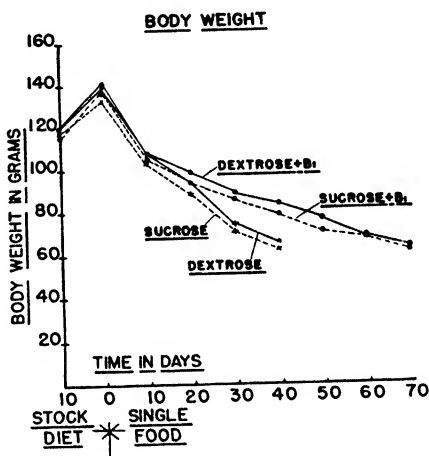


Fig. 1B

Fig. 1A. Graphs showing average daily running activity in 10-day periods for 11 rats on sucrose and thiamine, and 12 rats on dextrose and thiamine. The ordinates give the activity in number of revolutions of the drum, the abscissae time in days.

Fig. 1B. Graph showing body weight curves for the same four groups of rats.

initely less sucrose during the first two 10-day periods, after that they ate almost exactly the same amounts of the two sugars. When given access to thiamine hydrochloride the rats consistently ate from 12 to 20 per cent less sucrose than dextrose.

**Total fluid intake.** The total intake of fluid was essentially the same for all groups of rats, starting at a level near 24 cc. per day on the stock diet, and decreasing rapidly at first on the experimental diets, then more slowly until a flat level was reached at 4 to 5 cc. per day. See figure 3A.

**Thiamine hydrochloride intake.** Figure 3B shows the thiamine hydrochloride

<sup>4</sup> In previous experiments (3) it was reported that during the first 10 days on the single food diet the rats on dextrose ate 18 per cent less as measured in grams than they had during the previous 10 days of the sucrose diet, while the rats on sucrose ate 36 per cent less.

intake in micrograms per kilogram of food intake. During the first 30 days the sucrose-fed group of rats took considerably larger amounts of the thiamine

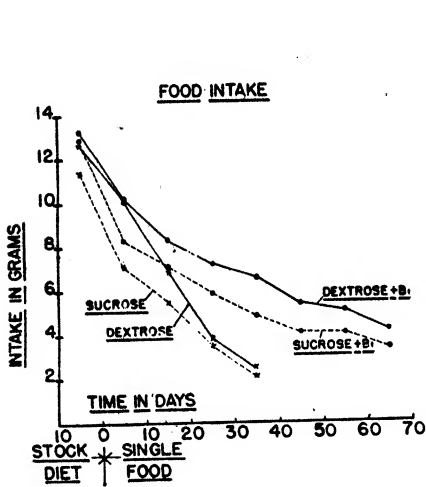


Fig. 2A

Fig. 2A. Graphs showing average daily food intake for 10-day periods for the four experimental groups of rats.

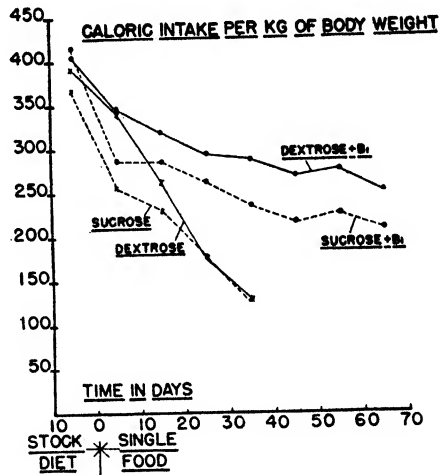


Fig. 2B

Fig. 2B. Graphs showing average daily food intake in calories per kilogram body weight for four experimental groups of rats.

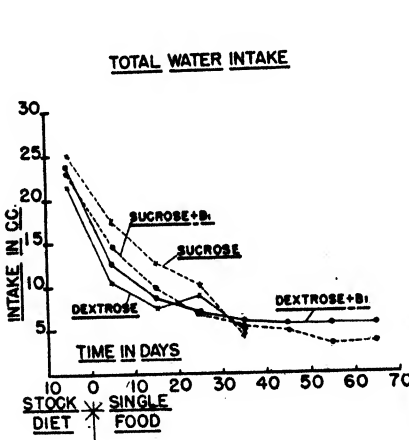


Fig. 3A

Fig. 3A. Graphs showing average total daily fluid intake (water alone or water plus thiamine solution) in 10-day periods.

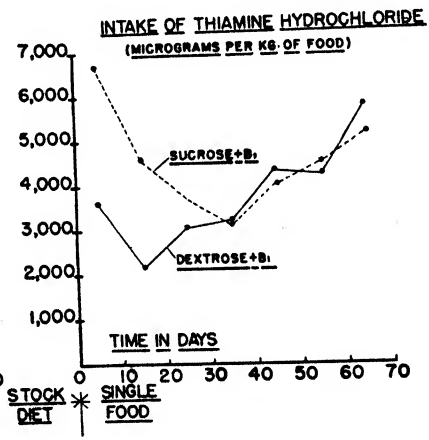


Fig. 3B

Fig. 3B. Graphs showing average daily intake of thiamine hydrochloride in micrograms per kilogram of food for 10-day periods for the two groups of rats which had access to the thiamine solution.

solution than did the dextrose-fed group. During this time, however, the sucrose-fed rats decreased their intake at a rapid rate while the other group

maintained their intake. The average intake was the same in both groups during the 30th to 40th day period and after that increased at the same rapid rate.

*Vaginal smears.* After showing one, or occasionally two, 4 to 5 day oestrous cycles on the single food sugars, all of the rats of both groups except one given dextrose remained in dioestrus until death. This one rat survived 54 days and showed constant cornification of the cells of the vaginal smears from the 35th day until it died.

With access to the thiamine solution rats given either dextrose or sucrose likewise showed dioestrous smears after 4 to 10 days on the single food diets. The rats given dextrose and thiamine hydrochloride showed dioestrous smears for 48 days on the average, then showed only constant cornification of the vaginal cells. In contrast, the vaginal smears of the rats on sucrose and thiamine solution began to show constant cornification after the 40th day on the average, that is, about 8 days sooner than the rats on dextrose and thiamine.

Constant cornification of the cells of the vaginal smears is one of the first and most reliable signs of vitamin A deficiency. It would appear therefore that on the sucrose and vitamin B<sub>1</sub> single food diet rats developed a vitamin A deficiency sooner than on the dextrose and B<sub>1</sub> diet and that this difference might account for the shorter survival time of the sucrose-fed rats. Further experiments were undertaken to answer this question.

*Effects of cod-liver oil on survival time, etc.* Five rats given access to sucrose, thiamine hydrochloride and cod-liver oil (in 30 cc. graduated inverted bottles) survived on the average 65 days, 9 days longer than without cod-liver oil. The rats took minimal amounts of cod-liver oil, so that it seems unlikely that the intake of fat affected the result. The daily intake for the first 60 days averaged 0.25 cc. That these rats were entirely devoid of signs of vitamin A deficiency is shown by the complete absence of cornified cells in the vaginal smears at all times. Further, that the effect produced on the vaginal smears and on survival time depended on the vitamin A obtained from the cod-liver oil, and not on the fat, is shown by the result of experiments in which we offered 7 rats access to cod-liver oil during the last 10 days on the stock diet but not at any time while on the single food diet. The vaginal smears of these rats, like those of the rats which had access to cod-liver oil while on the single food diet, did not show cornified cells at any time. Furthermore, their survival times averaged 74 days, almost 20 days longer than without any cod-liver oil at all, and exactly the same as that of the rats on dextrose and the thiamine hydrochloride solution.

Ten rats given dextrose, thiamine hydrochloride and cod-liver oil survived on the average 80 days, that is, 6 days longer than without cod-liver oil, and 6 days longer than the average survival time of the corresponding group of sucrose-fed rats. Like the rats on sucrose these rats did not show cornification of the vaginal smears at any time.

**DISCUSSION.** The results of these experiments show that, when offered as the sole source of nourishment, dextrose and sucrose appear to have almost exactly the same nutritive value. The survival time, body weight curves, and general spontaneous activity were essentially the same. The only difference



appeared to be that the rats ate slightly less sucrose than dextrose. Based on our observation, made under a variety of different conditions, that rats usually eat just as much of a given food as they are able to utilize, this result would seem to indicate that rats are not able to utilize as large amounts of sucrose as of dextrose. However, since they thrived as well on sucrose as on dextrose it may be an indication that rats are able to utilize sucrose slightly more efficiently than dextrose. This difference may also depend on the levulose component of the sucrose or on the necessity of hydrolyzing sucrose before its utilization.

Further experiments showed that when rats are carried much beyond the average survival time of rats on single foods, namely, 35 to 40 days, they develop vitamin A deficiency sooner on sucrose than on dextrose. This might mean either that more vitamin A is needed for the metabolism of sucrose or that in the metabolism of sucrose more vitamin A is destroyed. It may be mentioned in this connection that Bender, Ansbacher, Flanigan and Supplee reported that rats kept on a diet in which sucrose constituted the basal carbohydrate developed a marked dermatitis as early as five weeks after the start of the diet while rats kept on dextrin diet did not develop the dermatitis at any time (6).

It was further shown that the vitamin A obtained from cod-liver oil ingested by the rats in the 10-day pre-experimental period was stored in adequate amounts to prevent the appearance of signs of vitamin A deficiency for at least 74 days.

These experiments showed that when both groups of rats had access to cod-liver oil, the dextrose-fed rats lived slightly longer than did the sucrose-fed rats. It might be concluded from these experiments that the shorter survival times of the sucrose-fed rats resulted from the earlier development of vitamin A deficiency. That, however, other factors may play a part is shown by the observation that the dextrose and thiamine-fed rats, which showed signs of vitamin A deficiency as early as the 50th to 60th day, survived as long as did the rats on sucrose, thiamine, and cod-liver oil, which did not show signs of vitamin A deficiency at any time.

The levulose component of the sucrose may be responsible for the earlier appearance of the vitamin A deficiency on sucrose. More vitamin A may be needed for the utilization of levulose than dextrose. This deficiency may also account for the slightly smaller effect of thiamine hydrochloride on the utilization of sucrose.

It may be emphasized again that under the conditions of the single food experiments the rats did not develop signs of any dietary deficiencies over periods of 60 to 80 days except for those attributable to a lack of vitamin A, despite the absence in their diet of all the many needed minerals, vitamins, fatty acids, amino acids, etc. This is in marked contrast with the results which are obtained under conditions in which rats receive a diet complete except for one substance, vitamin or mineral, but mixed in fixed proportions allowing no choice of individual components. It is well known that rats develop signs of the lack of the one missing substance; in less than 10 days, for instance, in the case of magnesium. It would seem probable that in the single food experiments the rats are able to balance their caloric and vitamin intakes, that is to say they ingest as many

calories as they are able to utilize. In the normal full diet experiments in which the various minerals, vitamins, carbohydrates, fats, and proteins must be taken in fixed proportions, and no self-regulation is possible, higher caloric intake, and the resultant higher metabolism, may destroy reserves of those substances that cannot be obtained in needed amounts.

Finally attention may be called again to the great energy of the rats on diets limited to one carbohydrate. Even after 50 days the rats with access to the vitamin run from 6 to 18 miles per day. The low water intake of the rats on the single sugar either with or without thiamine is also to be noted. This confirms the observations made in previous single food experiments (3-5). Gamble has recommended dextrose as an emergency ration for men without access to drinking water (7).

#### SUMMARY

1. On a diet in which sucrose constituted the sole source of nourishment 11 female rats of an average age of 63 days and body weight of 133 grams survived on the average 37 days, while on a single food dextrose diet 21 female rats of an average age of 64 days and body weight of 137 grams also survived on the average 37 days. The two groups were equally active, showed the same rate of weight loss. A lower average daily intake of sucrose constituted the only measurable difference between the rats on the two sugars.

2. When offered access to a 0.02 per cent solution of thiamine hydrochloride the rats given dextrose lived 74 days while the rats given sucrose lived only 56 days. The rats on sucrose ate appreciably less food but for the first 30 days were as active as the rats on dextrose. After that they rapidly became less active.

3. Rats on a single food sucrose diet developed a vitamin A deficiency sooner than did rats on dextrose which may account for their decreased activity and shorter survival times. After elimination of vitamin A deficiency the rats on sucrose lived nearly as long as those on dextrose, had the same body weight curves, and were equally active.

4. At no time did rats on the single food diets develop any signs of dietary deficiency with the exception of the appearance of constant cornification of the vaginal cells. The fact that the rats could adjust their caloric intake to their ability to utilize the food probably explains this lack of dietary deficiencies.

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# SELF-SELECTION STUDIES ON COPROPHAGY AS A SOURCE OF VITAMIN B COMPLEX

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Many observers have noted that under conditions of dietary deficiency animals will eat their own feces or feces from other animals and that this ingestion of the feces prevents the appearance of deficiency symptoms (1, 2). Thus, for studies on the effects of dietary deficiencies the eating of feces, or coprophagy, must be rigidly eliminated. For purposes, however, of studies on the ability of rats to make beneficial dietary selections it becomes a phenomenon not to be eliminated but to be investigated in its own right (3). Hitherto only a few attempts have been made to single out eating of feces for special experimental study. Roscoe who collected feces from vitamin B deficient rats and returned them to their cages found that the rats ate from 40 to 100 per cent of the feces and lived for long periods of time (4). Guerrant and Dutcher collected feces from rats kept on diets in which either sucrose or dextrin constituted the source of carbohydrates and fed them to vitamin B deficient rats (5). The rats ate the feces from the dextrin group but not from the sucrose group, presumably because the sucrose did not support the intestinal bacterial synthesis of vitamin B while the dextrin did support this synthesis.

An attack on the problem has now been made with the self-selection technique. In the following experiments rats kept on a diet which lacked all components of the vitamin B complex were given access to feces from normal animals. We wished to determine whether the rats would eat the feces, and if so in what amounts and with what consistency over long periods of time, and the extent to which the ingestion of the feces would replace the entire vitamin B complex.

For these experiments the rats had access to one representative of each of the most important foodstuffs in purified or nearly purified form and in separate non-spillable containers. From the results of previous experiments it was known that most rats show normal growth on selections made from the following substances:

- |   |  |
|---|--|
| 1. Dextrose                             | 6. Sodium chloride 3 per cent          |
| 2. Olive oil                            | 7. Dibasic sodium phosphate 4 per cent |
| 3. Casein (vitamin free)                | 8. Potassium chloride 1 per cent       |
| 4. Dried brewer's yeast or liver powder | 9. Calcium lactate 2 per cent          |
| 5. Cod-liver oil                        | 10. Magnesium chloride 0.5 per cent    |
|   | 11. Tap water                          |

It will be noted that in this assortment of substances yeast or liver powder is the only source of the vitamin B complex. The rats did less well on liver powder

than on yeast in that fewer rats showed normal growth curves. When the rats refused to eat either the yeast or liver powder they lost weight at a rapid rate. Very few rats refused to eat the yeast powder, while a fair number refused to eat the liver powder, presumably because of its bitter taste.

Important for the present purposes is the observation made in some of the previous self-selection experiments that the appetites for carbohydrate, fat and protein depended on the components of the vitamin B complex which the rats received (6, 7). Without access to any of the components, that is, without yeast, the rats ate large amounts of fat, little or no carbohydrate, and no protein. With access to thiamine as the sole source of the vitamin B complex, the rats ate less fat, more carbohydrate, but still almost no protein. Progressively as the other components, riboflavin, niacin, pyridoxine, pantothenic acid, and choline chloride were made accessible in separate containers, the rats ate larger amounts of carbohydrate, more protein, and smaller amounts of fat; and progressively the body weight curves more closely approached the normal. Thus the proportions of the carbohydrate, fat, and protein intakes give a rough indication of the presence of the aforementioned six components of the vitamin B complex in the diet. Accordingly when on the self-selection diet the rats have access to yeast, which contains all of the six vitamin B components at least in small amounts, most rats show a great carbohydrate and protein appetite and small appetite for fat.

In the following self-selection experiments yeast or liver powder were omitted from the ten substances offered for choice and were replaced by feces collected from normal rats. This meant that the only vitamin B that could have been present in the diet must have been contained in the feces. Comparisons were made of the body weight curves and of the proportions of the carbohydrate, fat, and protein intakes when yeast, liver, or feces constituted the sole source of the vitamin B complex.

**METHODS.** Previous reports contain a full description of the individual cages and the technique used for self-selection experiments. Each cage was equipped with a screened bottom and contained three non-spillable cups for the solids and eight graduated inverted bottles for the fluids.

The intake of each substance was recorded daily. The bottles were cleaned and refilled twice weekly. The animals were weighed weekly.

The experiments were carried out in three series. In the first the rats were given access for a period of 50 days to the assortment of substances listed above in which yeast constituted the sole source of the vitamin B complex. Then for a period of 54 days feces from normal rats replaced the yeast. After that the feces were omitted leaving the diet entirely free from all vitamin B. In the second series the conditions were essentially the same except that the rats had access first to liver powder rather than to yeast. They had access to liver for 38 days and to feces for 65 days. In the third series the rats were given access to feces from normal rats for a period of 40 days. Then for a period of 66 days yeast replaced the feces. After that the yeast was omitted.

The feces were collected daily from healthy young adult rats which were given our stock diet<sup>1</sup> and were kept in screened bottom cages. The feces were ground in a meat grinder before they were given to the rats. Each day all of the feces remaining from the previous day were removed.

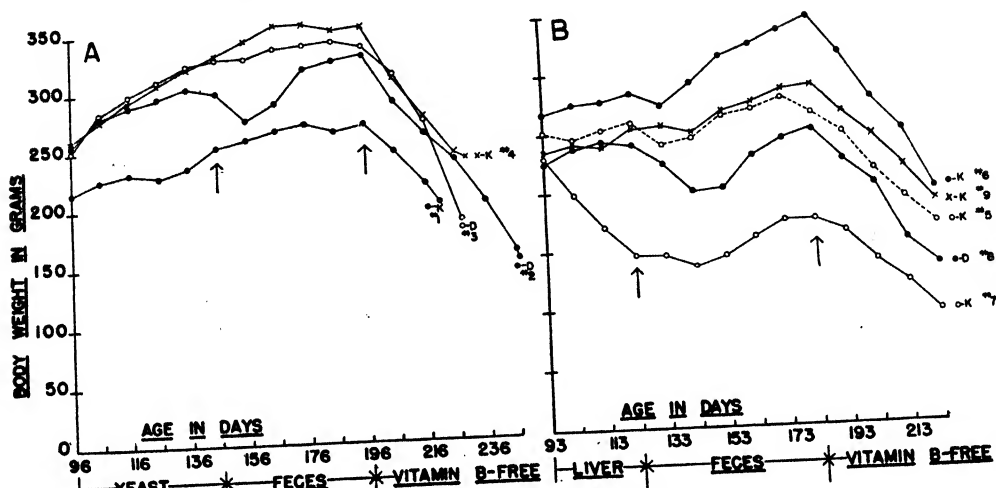


Fig. 1A. Body weight curves for 4 rats of first series. 1B. Body weight curves for 5 rats of second series.

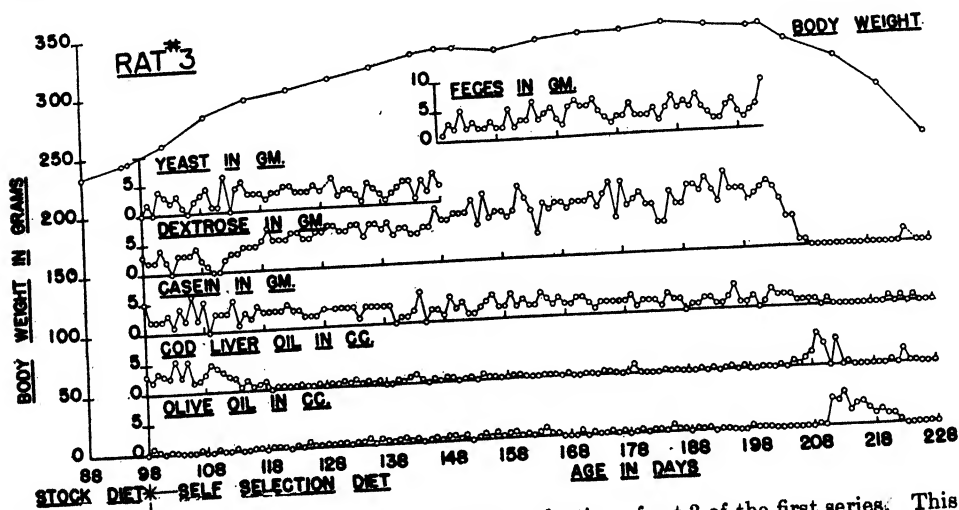


Fig. 2. Body weight curves and daily dietary selection of rat 3 of the first series. This rat had access to yeast for 50 days (ages 98-148 days); then to feces for 54 days (ages 149-202 days); after that it had access to no vitamin B (ages 203-228 days).

**RESULTS. First Series. Body weight.** Figure 1A shows the body weight curves of the four rats used in this series. Until these rats were 96 days of age

<sup>1</sup> This diet contained graham flour 72.5 per cent, casein 10.0 per cent, butter 5.0 per cent, skim milk powder 10.0 per cent, calcium carbonate 1.5 per cent, sodium chloride 1.0 per cent.

they received only our regular stock diet. They were then placed on the self-selection diet with access to the 11 different substances. For the first 50 days yeast represented all of the components of the vitamin B complex; for the next 54 days yeast was omitted and replaced by the freshly ground feces; after that the feces were removed, leaving the rats on a diet which lacked all components of the vitamin B complex. On the yeast diet two rats gained at a normal rate; two at a reduced rate. When changed to the feces diet, three rats continued to gain at approximately their previous rate, one rat lost weight temporarily and then quickly caught up with the other rats. After the removal of the feces all 4 rats lost weight at a rapid rate. Two rats died after 38 days and 48 days on the B-free diet respectively. The other two were killed after 25 and 32 days respectively. Thus, when given access to feces the rats grew quite as rapidly as on yeast, and when deprived of feces they lost weight at a very rapid rate.

*Dietary selections.* Figure 2 shows a typical self-selection record for one of the rats. The chart shows the daily intake of dextrose, casein, cod-liver oil, and olive oil during the three periods when the rat had access to yeast, feces, and no vitamin B respectively. It also shows the body weight curve. During the 50-day period on yeast the intake of the various substances remained fairly constant, except for a gradual increase in the intake of dextrose and simultaneous decrease in oil consumption. At the end of this period the rat ate about 2 grams per day of yeast, about 5 grams of dextrose, 2 grams of casein, and almost no cod-liver oil or olive oil. When feces replaced the yeast there was almost no noticeable change in the body weight curve. The rat ate the feces at once and after a few days in slightly larger amounts than it had previously eaten the yeast. Its dextrose intake increased while the casein intake decreased slightly. The rat still took only minimal amounts of the oils. When the feces were removed, leaving no source of vitamin B, the animal began losing weight at once, stopped eating dextrose and casein, and started at first to take cod-liver oil, later olive oil in large amounts. Still later when the rat was failing generally, it also stopped taking fat.

*Intake of yeast and feces.* The first two columns in table 1 show the average daily intake of yeast and feces for the 4 rats for the last 20 days of the period on yeast and on feces. The yeast intake averaged 2.2 grams; the feces intake 4.5 grams. Thus, the rats ate about twice as much of the feces as of the yeast.

Almost at once after the feces were made available the rats ate them freely and in large amounts. They continued to eat constant and large amounts throughout the experimental periods. They seemed to accept the feces as readily or even more readily than yeast and definitely more readily than the liver powder.

From observations made on the daily output of feces of rats kept on our stock diet we were able to determine how many rats were required to supply the feces eaten daily by these rats. The feces of 28 adult rats were collected and weighed daily for 15 days. Their individual daily output averaged 1.6 grams with only minor variations from rat to rat. Thus the 4.5 gram intake of feces per rat represented the output of 2.8 rats on the stock diet.

*Intake of carbohydrate, protein and fat.* Table 1 also gives the average daily

intake of dextrose, casein, and of olive oil and cod-liver oil for the 4 rats. The average daily intake of dextrose increased from 6.0 grams for the last 20 days on the yeast diet to 8.0 grams for the last 20 days on the feces diet. From the 11th to 30th days on the vitamin B free diet (no yeast or feces) the dextrose intake dropped sharply to 0.4 gram. Casein intake remained essentially the same on the feces as on the yeast diet but decreased almost to zero during the vitamin B free period. The combined intake of olive oil and cod-liver oil decreased during the period when the rats had access to feces but increased very definitely during the vitamin B free period. All 4 rats showed essentially the same changes in appetite.

Table 2 shows that the average daily total caloric intake was 51.7 during the yeast period and 49.5 during the feces period and dropped to 20.4 after removal

TABLE 1  
*Average daily food intake for last 20 days on yeast, liver, and feces diet, and for 11-30 days on vitamin B-free diet*

	SOURCES OF VITAMIN B COMPLEX IN GRAMS		DEXTROSE IN GRAMS			CASEIN IN GRAMS			OLIVE-OIL AND COD-LIVER OIL IN CC.		
	Yeast	Feces	Yeast diet	Feces diet	Vitamin B-free	Yeast diet	Feces diet	Vitamin B-free	Yeast diet	Feces diet	Vitamin B-free
First series (4 rats)	2.2 (0.9-3.3)	4.5 (3.8-5.7)	6.0 (2.4-10.6)	8.0 (5.7-10.9)	0.4 (0.2-0.5)	2.7 (2.1-4.2)	2.9 (1.7-4.3)	0.2 (0.1-0.3)	1.2 (0.4-2.3)	0.7 (0.3-1.3)	2.2 (1.6-2.8)
Second series (5 rats)	Liver		Liver diet			Liver diet			Liver diet		
	2.2 (0.1-3.1)	5.4 (3.7-7.2)	5.7 (0.6-9.5)	8.7 (7.3-9.9)	1.4 (0.5-2.0)	0.5 (0.1-0.9)	2.0 (0.1-3.4)	0.3 (0.2-0.6)	0.9 (0.2-1.6)	0.3 (0.1-0.5)	1.6 (0.6-2.4)
Third series (5 rats)	Feces	Yeast	Feces diet	Yeast diet		Feces diet	Yeast diet		Feces diet	Yeast diet	
	3.0 (1.6-3.9)	2.4 (1.2-3.8)	6.4 (1.3-8.3)	4.4 (1.8-6.1)	1.7 (0.3-5.4)	1.4 (0.6-2.1)	1.6 (0.1-3.9)	0.2 (0.1-0.3)	0.6 (0.3-1.8)	0.8 (0.3-1.9)	1.1 (0.5-1.5)

of the feces. The table also shows the percentage intake of carbohydrate, fat, and protein. The intake of carbohydrate increased on the feces diet from 52.8 to 64.7 per cent while the fat intake decreased sharply and the protein intake decreased only slightly. After removal of the feces the carbohydrate intake dropped from 64.7 to 7.9 per cent while the intake of fat increased from 12.7 to 88.2 per cent and the intake of protein decreased from 22.6 to 3.9 per cent.

*Intake of minerals.* Table 3 gives the average daily intake record of the five mineral solutions for the rats in the three series of experiments. In the first series it was found that the rats decreased their calcium lactate intake when changed to feces, and increased it again when the feces were removed. The intake of sodium phosphate showed just the reverse changes. The intake of potassium chloride decreased on the feces diet and also when the feces were removed. The intake of magnesium chloride was very high on both yeast and

feces and showed a very sharp drop after removal of the feces. The intake of sodium chloride gradually decreased.

*Second Series. Body weight.* Figure 1B shows the body weight curves of the 5 rats used in the series in which the rats had access to powdered liver in place of yeast. None of the rats grew as well as on yeast. Four showed only a slight gain during the 38 day period and one showed a marked loss. During the 65 day interval in which the feces replaced the liver, the 4 rats which had previously shown a slight gain lost weight temporarily, then gained at a more rapid rate than on liver. The fifth rat stopped losing weight, maintained its weight at a flat level for about 40 days, then started to gain at an almost normal rate. When the feces were no longer offered to them, all of the rats lost weight at a constant and very rapid rate.

TABLE 2

	AVERAGE DAILY TOTAL CALORIC INTAKE-LAST 20 DAYS OF EACH PERIOD	CALORIC PERCENTAGES OF		
		Carbohydrate	Fat	Protein
First series (4 rats)				
Yeast period (50 days).....	51.7	52.8	19.8	27.4
Feces period (54 days).....	49.5	64.7	12.7	22.6
Vitamin-B-free period*.....	20.4	7.9	88.2	3.9
Second series (5 rats)				
Liver period (38 days).....	40.8	55.2	25.2	19.6
Feces period (65 days).....	45.5	76.5	5.9	17.6
Vitamin-B-free period*.....	20.3	27.6	66.5	5.9
Third series (5 rats)				
Feces period (40 days).....	36.6	69.9	14.8	15.3
Yeast period (66 days).....	38.0	56.0	17.5	26.5
Vitamin-B-free period†.....	15.4	44.1	51.4	4.5

\* 10th to 30th days.

† 30th to 50th days.

*Intake of liver and feces.* The second part of table 1 summarizes the results. The daily intake of liver averaged 2.2 grams and intake of feces averaged 5.4 grams which is almost  $2\frac{1}{2}$  times the intake of the liver. On the basis of an average output of 1.6 grams of feces per day for a rat on the stock diet, it would require 3.4 normal adult rats to supply this amount of feces. It will be noticed that one rat ate practically no liver. This is also the rat which showed the large loss in weight.

*Intake of carbohydrate, casein and fat.* The second part of table 1 gives the average daily intake of dextrose, casein, olive oil and cod-liver oil for the 5 rats for the last 20 days of each of the three periods. The changes were essentially the same as in the first series. The average daily intake of dextrose increased from 5.7 grams on liver to 8.7 grams on feces and decreased to 1.4 on the un-supplemented diet. The casein intake increased from 0.5 gram to 2.0 grams on feces and decreased to 0.3 on the vitamin B free diet. The fat intake decreased



from 0.9 gram on liver to 0.3 gram on feces and increased to 1.6 grams when no vitamin B was available to the animals.

The dietary selections of the rat which consistently refused to eat the liver powder are worthy of special comment. Figure 3 shows the liver, feces, dextrose, casein, cod-liver oil and olive oil intake of this animal, also the weekly records of body weight. This rat was placed on the self-selection diet at the age of 93 days. During the following 33 days it ate no liver powder and showed the constant sharp loss in body weight typical for rats of this age on a vitamin B free diet. Progressively during the first twenty days it ate less dextrose, more fat, and no casein. After that when the animal began to fail it also ate less fat. At an age of 132 days feces were made available in place of liver powder. They were offered in the same container. On the very first day the rat ate a large amount

TABLE 3

*Average daily intake of mineral solution in cubic centimeters for last 20 days on yeast, liver, feces and for 10-20 day period on vitamin B-free diet*

	CALCIUM LACTATE (2%)			SODIUM PHOSPHATE (4%)			POTASSIUM CHLORIDE (1%)			MAGNESIUM CHLORIDE (0.5%)			SODIUM CHLORIDE (3%)		
	Yeast diet	Feces diet	Vitamin B-free	Yeast diet	Feces diet	Vitamin B-free	Yeast diet	Feces diet	Vitamin B-free	Yeast diet	Feces diet	Vitamin B-free	Yeast diet	Feces diet	Vitamin B-free
First series (4 rats)	2.6	0.9	4.5	4.5	12.1	4.3	4.6	2.7	1.6	14.7	12.0	3.4	4.2	2.1	2.0
	Liver diet			Liver diet			Liver diet			Liver diet			Liver diet		
Second series (5 rats)	1.9	1.2	2.6	2.1	6.1	3.7	1.8	2.0	2.9	5.8	5.2	3.0	3.5	2.6	3.2
	Feces diet	Yeast diet		Feces diet	Yeast diet		Feces diet	Yeast diet		Feces diet	Yeast diet		Feces diet	Yeast diet	
Third series (5 rats)	1.5	2.7	3.1	4.4	5.5	9.3	1.8	2.6	4.4	6.6	3.6	3.9	3.5	3.9	4.0

of feces, approximately 5 grams, and continued to eat as large or even larger amounts during the following 65 day period. Of special interest is the sudden return of the appetite for dextrose that occurred on the day after feces were offered. Over a 20 day period the daily dextrose intake increased from 0 to 10 grams. The rat stopped losing weight but did not start to make a definite gain until approximately 30 days later. After that it recovered a part of its original weight loss. After the feces were removed the dextrose intake gradually decreased again and the cod-liver oil intake increased. The failure of the rat to take casein at any time after the feces were made available was most probably due to the advanced stage of emaciation reached by the rat during the vitamin B free period while the rat did not eat liver powder.

Table 2 shows that the average daily total caloric intake increased on the feces diet from 40.8 to 45.5 and decreased to 20.3 after removal of the feces. The

percentage intake of carbohydrate increased from 55.2 on the liver diet to 76.5 on the feces diet and dropped to 27.6 after removal of the feces. Fat intake showed the opposite changes. It decreased from 25.2 on the liver diet to 5.9 on the feces diet and increased to 66.5 after removal of the feces. The protein intake decreased only after removal of the feces.

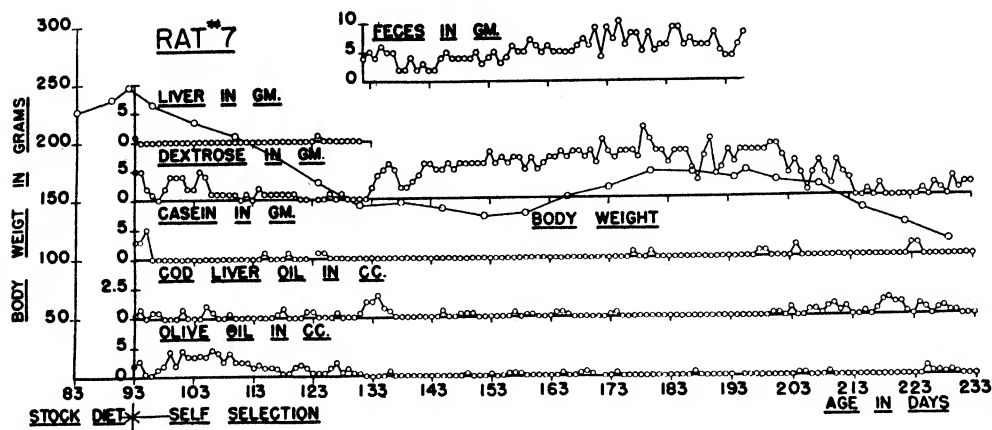


Fig. 3. Body weight curve and daily dietary selection of rat 7 of the second series. The rat had access to liver powder for 38 days; then to feces for 65 days, and thereafter to no vitamin B.

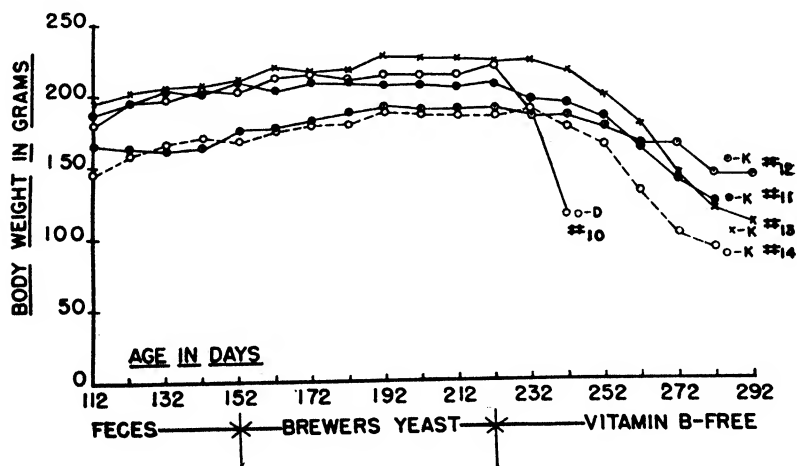


Fig. 4. Body weight curves of 5 rats of the third series.

*Intake of minerals.* The changes in appetite for the mineral solutions were less constant than in the first series, but in general they were similar. See table 3.

*Third Series.* In this series the rats started on the feces diet and were later changed to the yeast diet. The results are not strictly comparable to those obtained on the 9 males of the first and second series, since the 5 rats used were females which weighed considerably less and furthermore were about 20 days older.

*Body weight.* Figure 4 shows the body weight curves of these 5 rats. During the 40 days on the feces diet these rats gained at a slow but steady rate. During the 66 days on the yeast diet they continued to gain at approximately the same rate. The change from feces to yeast was not reflected in these curves. After removal of the yeast 4 of the rats began to lose weight but less rapidly than after the removal of feces in the first and second series. One rat lost weight very rapidly and died after 10 days.

*Feces and yeast intake.* The third part of table 1 summarizes the results of these experiments. The feces intake averaged 3.0 grams for the last 20 days on the feces diet, while the yeast intake for the last 20 days averaged 2.4 grams. According to our previous calculations the feces intake represents the average daily output of approximately 2 rats. It will be noticed that one animal ate only half as much of the feces as did the other four.

*Intake of dextrose, casein and oils.* Table 1 summarizes the results. The dextrose intake was higher on the feces than on the yeast diet but decreased only a small amount after removal of the yeast. The intake of fats remained very low throughout and failed to show the large increase present in the first and second series after removal of the feces. The average for the rat which ate the small amount of liver deviated considerably from those of the other animals. In all of the animals the changes in appetite which are characteristic for the vitamin B deficiency did not appear until after 30 days on the vitamin B free diet. This is in keeping with the observation that these animals lost weight at a less rapid rate than did the rats of the first and second series during the vitamin B free period.

Table 2 shows that the average daily caloric intake remained low in all 5 rats on the feces and yeast diets and decreased after removal of the yeast. Carbohydrate averaged 69.9 per cent on the feces diet, decreased to 56.0 per cent on the yeast diet, and decreased to 44.1 per cent after removal of the yeast. Fat intake showed the reverse changes on the feces diet and yeast diet but also increased after removal of the yeast. The protein intake increased definitely on the yeast diet and decreased sharply after removal of the yeast.

*Intake of minerals.* The third part of table 3 summarizes the results. The individual differences were too great to permit any conclusions to be drawn.

**DISCUSSION.** The results of these self-selection experiments on coprophagy showed that rats on a vitamin B deficient diet ate large amounts of feces freshly collected from normal rats. Measured in grams they constituted  $\frac{1}{4}$  to  $\frac{1}{3}$  of the entire diet. They started to eat the feces at once, definitely more readily than they ate liver powder, and quite as readily as they ate yeast powder. Particularly noteworthy was the constancy of the intake from day to day during the 40 to 50 day experimental periods.

That the rats on the feces diet grew or maintained their body weight as well as the rats on the yeast diet makes it seem very likely that the feces must contain the same growth promoting components of the vitamin B complex which are present in yeast.

The effects produced by the ingestion of feces on the carbohydrate, fat and

protein appetite also indicate that the feces probably contained not only thiamine but riboflavin, niacin, pyridoxine, pantothenic acid and choline chloride. That in most instances the rats on feces had a higher carbohydrate appetite than the rats on yeast suggests that feces may contain even greater amounts than yeast of some of the vitamin B components, particularly of thiamine.

The comparison, however, of the results of the first and third series brings out a difference between yeast and feces. In the first series omission of the feces from the substances offered for choice after the rats had had access to them for 54 days was followed by a sharp decrease in body weight and death within a few weeks' time. In contrast in the third series the omission of yeast after the rats had had access to it for 66 days was followed by a slower decrease in body weight. Thus it would appear that on the yeast diet the rats were better able to store some of the vitamin B components.

It may be noted here that we do not know whether the rats ate the feces exclusively to supply the needed vitamin B. It is possible also that they ate the feces for other substances, some of them which may not even be present in yeast or liver powder. That under these conditions in which a number of other substances were available at the same time rats ate the feces at once and did not have to be starved into eating them, indicates that the feces do not contain any bitter tasting or otherwise unpleasant tasting and probably toxic substances. They differ in this respect from yeast and particularly liver powder which for some rats must have such a bitter taste that they refuse to eat them although in doing so they develop a serious vitamin B deficiency.

That the feces of rats contain vitamin B we know from the observations made by numerous workers starting with those of Osborne and Mendel who conducted the first experiments on the effects of a diet consisting in part of feces (8). Later experiments were carried out by Steenbock, Sell and Nelson (1), Dutcher and Francis (9), Heller, McElroy and Garloch (10), Smith, Cowgill and Croll (11), Roscoe (4), Guerrant and Dutcher (2), Kennedy and Palmar (12), Moore, Phymate and White (13), and others. The last named workers reported that the feces from 10 rats on a diet supplying 1 per cent yeast were found sufficient to keep the majority of 25 young rats alive for over eight months when used as the sole source of vitamin B. The use of wheat germ oil in the diet however probably invalidates their results.

The source of the vitamin B contained in the feces still remains doubtful. The results of our self-selection experiments throw no new light on this question. We do not know whether the vitamin B in the feces represented an undigested excess which passed through the intestinal tract unchanged or whether it was synthesized in the intestinal tract by bacteria. Most of the evidence at hand however favors the latter explanation.

#### SUMMARY

1. Rats kept on a self-selection diet completely lacking all components of the vitamin B complex ate 3 to 5 grams per day of feces collected from normal adult

rats. The daily output of feces from 2 to 4 normal adult rats supplied all of the needed vitamin B components for one rat on an otherwise B deficient diet.

2. Feces-fed animals continued to grow at normal rates and showed no signs of specific deficiency. Their appetites for carbohydrate, fat and protein were essentially the same as they had been when given access to yeast or liver powder.

3. When no longer given access to feces the rats at once lost weight at a rapid rate. They showed the changes in appetite which are characteristic of vitamin B deficiency, namely, a substitution of fat for most of the carbohydrate and protein previously taken.

4. Thus it is concluded that for at least 40 to 50 days feces satisfactorily replaced all the components of the vitamin B complex found in yeast or liver powder.

5. That the rats ate the feces at once and in large amounts and over long periods of time without any ill effects would indicate that the feces did not contain any bitter tasting or toxic substances.

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# THE EFFECT OF CALCIUM CONCENTRATION ON THE PROTHROMBIN TIME OF DOGS TREATED WITH DICUMAROL

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The measurement of plasma prothrombin by the determination of Quick's prothrombin time has become a standard laboratory procedure. Quick (1939) found that while control of the calcium concentration is essential, yet it can be varied over a relatively wide range without any significant effect on the prothrombin time. In his earlier description of the method he used 0.040 M  $\text{CaCl}_2$ . Later he changed this to 0.025 M, but states that this does not significantly change the prothrombin time. Stewart and Pohle (1938) suggest the determination of the optimal calcium concentration for each sample but Quick denies that this procedure in any way improves the accuracy of the method. In connection with studies in this laboratory using dicumarol, however, it has been found that the long prothrombin time resulting from the administration of this substance is very sensitive to changes in calcium concentration.

**METHODS.** Dogs were given 2.5 or 5.0 mgm./kgm. of dicumarol. We are indebted to the Charles H. Frosst Co., Montreal, for a supply of the material. This was dissolved in physiological saline immediately before use by adding one or two drops of 5 N NaOH and was injected intravenously. The plasma prothrombin time, plasma clotting time, the blood prothrombin time and blood clotting time were then measured daily; the plasma prothrombin time and plasma clotting time were measured for a series of concentrations of calcium chloride.

The plasma prothrombin time was determined according to Quick (1940), using 0.1 cc. of thromboplastin extract from acetone-extracted rabbit brain + 0.1 cc. calcium chloride solution + 0.1 cc. oxalated plasma. The plasma clotting time was determined with 0.1 cc. saline + 0.1 cc. calcium chloride solution + 0.1 cc. plasma. The clotting time for each sample was determined with each of 0.25, 0.1, 0.05, 0.025, 0.01, 0.005, 0.0025, 0.001 M calcium chloride solutions. The blood prothrombin time was measured by the method of Ziffren, Owen, Hoffman and Smith (1939) with 0.2 cc. of blood added to 0.1 cc. of thromboplastin. At the same time, 0.3 cc. blood was added to a second tube to determine the blood clotting time. All determinations of clotting times were conducted in 8 mm. test-tubes placed in a constant temperature bath at 37°C. and the end point taken as the time at which the plasma or blood no longer flowed on tipping the tube. The final concentration of oxalate in the plasma was 0.01 M.

**RESULTS.** The results of a typical experiment are shown in figure 1. Before dicumarol administration, the same prothrombin time (20 sec.) is obtained for calcium concentrations ranging from 0.1 M to 0.01 M. Decreasing the calcium

concentration to 0.001 M increased the prothrombin time to 56 sec. After giving dicumarol the prothrombin time lengthened, reaching a peak value of 125 sec. on the third day. Of greater significance is the fact that the calcium concentration now has a very marked effect on the prothrombin time. On the second day with a prothrombin time of 60 sec. with 0.05 M Ca, this is lengthened to 110 sec. with 0.025 M and 150 sec. with 0.10 M. On the third day, measurable prothrombin times were only obtained with 0.05 (145 sec.) and 0.025 M (170 sec.). No clotting was observed with concentrations above and below this.

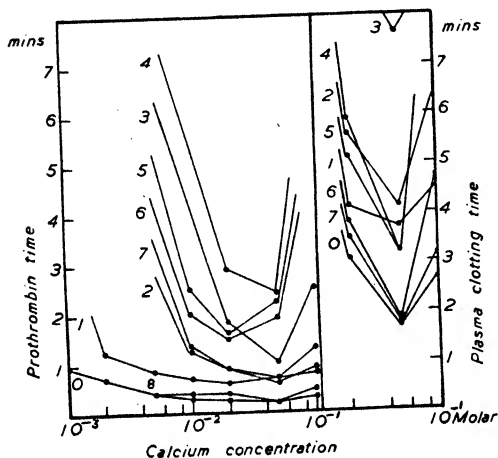


Fig. 1

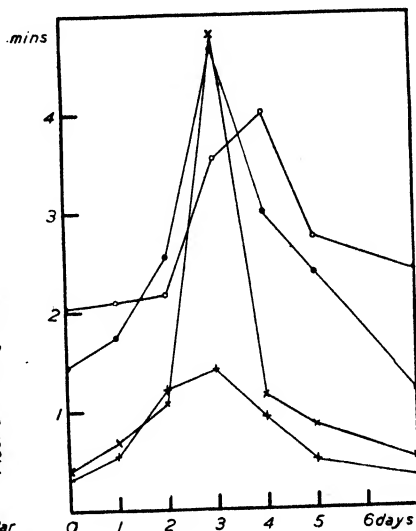


Fig. 2

Fig. 1. The effect of calcium concentration on prothrombin time and plasma clotting time after dicumarol. Dog 2. Five milligrams dicumarol/kgm. 0, 1, 2, . . . 8 days after dicumarol. No clot observed with the values of calcium concentration not plotted on the 1st to 7th day.

Fig. 2. Changes in clotting time, prothrombin time, etc., after dicumarol. Dog 1. Five milligrams dicumarol/kgm. ●—● clotting time of blood. ○—○ clotting time of plasma with optimal Ca. ×—× blood prothrombin time. +—+ plasma prothrombin time with optimal Ca.

With gradual return of the prothrombin time to normal, the calcium curve likewise gradually returned to the normal. It can be observed that there was some shift of the optimum value, as it was 0.05 M for the 2nd, 3rd, 6th, 7th and 8th days and 0.025 for the 1st, 4th and 5th days. This shift was not significant as it was not found consistently with other animals.

The normal plasma clotting time is even more affected by changes in calcium concentration than the plasma prothrombin time. The optimum appeared to be about 0.05 M and no clotting occurred with 0.25 and 0.01 M Ca, i.e., exact equivalence of calcium and oxalate (0.01 M) was not sufficient to produce clotting but rather a five-fold concentration was necessary to provide optimal calcium. After dicumarol, the clotting time was lengthened and here again

clotting could only be obtained at calcium concentrations close to the optimum. Since the plasma was 0.01 M with respect to oxalate, exact equivalence at 0.01 M should have provided excess calcium equivalent to the 10 mgm. per cent present initially or 0.0025 M. It is interesting that the lowest clotting and prothrombin times were obtained with 0.05 M, which would mean an excess of 0.04 M calcium added.

In figure 2 are shown the changes in clotting time, prothrombin time, etc., when the values for the optimal calcium concentration are determined. The clotting times for blood and plasma agree fairly closely and likewise the prothrombin times for blood and plasma. The long blood prothrombin time on the third day is of interest. Thus in another dog, 5 mgm./kgm. raised the prothrombin time to 54 sec. on the second day, 150 sec. on the third day, while the corresponding blood prothrombin times were 90 sec. and 170 sec. Further, the effect appeared to be proportional to the degree of hypoprothrombinemia. As shown above, the calcium concentration to give the shortest plasma prothrombin time with oxalated plasma is ten times the normal concentration in plasma, and therefore the long blood prothrombin time is probably due to the lower physiological calcium concentration. This was tested by adding various concentrations of calcium to the blood prothrombin test. Blood prothrombin time without calcium was 50 sec., with 0.001 M Ca was 31 sec., with 0.01 M Ca was 30 sec., with 0.025 M Ca was 27 sec., with 0.05 M Ca was 38 sec. and with 0.1 M Ca was 50 sec.

The question arises as to whether this calcium effect is due to the lowered prothrombin concentration in the blood after dicumarol or is due to some other factor. This was tested by diluting normal oxalated plasma with oxalated saline and determining the prothrombin time at various concentrations of calcium. It was found that dilution of plasma as much as 1:50 (giving a prothrombin time of 2 min. 50 sec.) did not change the calcium curve from that of undiluted plasma. A 1:100 dilution failed to clot with 0.001 M calcium but the calcium effect was not changed at other concentrations. In a second dog, with a 1:10 and 1:20 dilution of plasma, there was some increase in prothrombin time with the highest and lowest calcium concentrations, but this was slight compared to the dicumarol-treated animals with the same prothrombin times for optimal calcium concentrations. Thus a 1:10 dilution of plasma gave a 78 sec. prothrombin time, which was raised to 80 sec. by 0.001 M and 78 sec. by 0.1 M calcium chloride.

Quick (1941) reported that the plasma prothrombin concentration varied with the species. As this is an alternative method for varying prothrombin concentration and without a parallel dilution of fibrinogen, etc., similar calcium-prothrombin time curves were established for different species. These are shown in figure 3. It was found that while the prothrombin times of the species studied are relatively close together, the effect of calcium varied with the species. Thus while dog plasma showed no variation in prothrombin time with calcium concentration from 0.1 M to 0.0025 M, the rat plasma gave a prothrombin time of 110 sec. with 0.1 M, 26 sec. with 0.05 M and no clot with 0.0025 M. There was a greater difference between species in the plasma clotting time, and the



samples with the longer plasma clotting time were the ones which were most sensitive to changes in calcium concentration.

**DISCUSSION.** The importance of this effect of calcium on the prothrombin time is obvious. Witts (1941), in particular, has emphasized the discrepancies in prothrombin determinations in the dicumarol-treated animal. He points out that in the data of a number of investigators the prothrombin time is longer than the clotting time of the blood at the peak of the dicumarol effect. It is probable that this discrepancy has been due to the calcium concentration not being optimal. Thus, Quick (1944) states that he has never observed a discrepancy.

Again, the finding that the calcium concentration in the blood is not optimal, so that at the peak of the effect the blood prothrombin time is longer than the "plasma" prothrombin time, is of great significance. Quick has emphasized that the importance of the prothrombin time is not that it measures the amount of

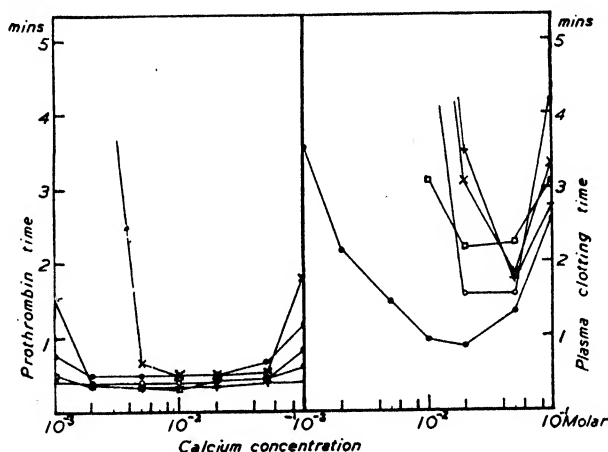


Fig. 3. The effect of calcium concentration on the prothrombin time of plasma from various species. ●——● Cat. ○——○ Rabbit. ×——× Rat. +——+ Dog. □——□ Human.

prothrombin present, but rather that it measures the changes in the clotting system corresponding to clinical observations but not detected by the clotting time. It would appear from this standpoint that determinations of the *blood* prothrombin time, in which the calcium concentration is that of the blood *in vivo*, would be more advisable than the usual determination of prothrombin time with oxalated plasma. Certainly the slight increase in "plasma" prothrombin time in figure 2 (from 25 sec. to 85 sec.) does not suggest the serious hemorrhagic tendency present and which was indicated when the blood prothrombin time rose to 4 min. 46 sec.

A serious discrepancy between the "plasma" prothrombin time and the results of the two-stage titration of prothrombin according to the technique of Smith, Warner and Brinkhous (1934) occurs when the methods are applied to the blood of different species. Correcting the "plasma" prothrombin time for the optimal calcium concentration did not improve the percent prothrombin calculated from it, so that this discrepancy is not due to the calcium effect.

Apparently the effect of calcium reported here is not due simply to the longer clotting time with thromboplastin when the prothrombin concentration is low. In general, the influence of the concentration of any factor on the clotting time is found to be on a logarithmic scale. Hence, the effect increases with increased clotting time, and it is not possible to conclude that a second factor (in this case the effect of calcium concentration) is exerting a different action, simply because of the greater effect at the new base line. However, the results obtained on diluting the plasma prove that the longer clotting time is not the factor responsible for the calcium effect.

The effect of changes in calcium concentration on the clotting system is usually considered to be due to two different actions. The effect on lowering the concentration below the optimum is thought to be due to a decrease in the concentration of the hypothetical calcium-protein complex involved in the clotting process, whereas the inhibitory effect of excess calcium chloride is thought to be due simply to the known action of divalent neutral salts. However, the failure to duplicate the effects of calcium concentration by diluting normal plasma shows that this cannot be the explanation of the effects observed. Dilution will both decrease the concentration of the calcium-protein complex, and increase the inhibitory action of salts (cf. Jaques and Mustard, 1940). Actually, when tested with calcium concentrations which are not optimal, the prothrombin in dicumarol plasma fails to exhibit the activity of the normal prothrombin system. Not only is there a quantitative but there is also a qualitative change in prothrombin following dicumarol. Many experiments of significance in the explanation of various aspects of clotting are suggested by the findings reported, and we plan to continue our studies of this subject.

#### SUMMARY

The clotting time and prothrombin time with whole blood, and the clotting time and prothrombin time of oxalated plasma on addition of various concentrations of calcium, were determined on dogs treated with dicumarol. Normal plasma gave very little variation in prothrombin time with calcium concentrations from 0.005 to 0.10 M. Dicumarol plasma shows a definite optimal calcium concentration of 0.025 M and concentrations above and below this value gave very much longer clotting times. The difference between these times and those with optimal calcium concentration increased up to the peak of the dicumarol effect and decreased as the prothrombin returned to its normal level. This effect of calcium on dicumarol plasma is not duplicated by diluting normal plasma to give the same prothrombin time with 0.025 M calcium. Differences between species were observed regarding the effect of varying calcium concentration on normal prothrombin time. Calcium concentration has an even more marked effect on the plasma clotting time. The blood prothrombin time in a highly dicumarinized animal can be reduced by adding more calcium.

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# A COMPARISON IN INTESTINE AND LEG OF THE REFLEX VASCULAR RESPONSE TO CAROTID-AORTIC CHEMORECEPTOR STIMULATION<sup>1</sup>

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Reflex vascular reactions initiated at the carotid and aortic bodies are assumed to be generalized reactions which, co-operating closely with the mechanisms for respiratory control, aid in distributing the cardiac output in accordance with changing respiratory conditions. In the dog the efferent pathway for these reflexes includes only thoracolumbar autonomic fibers (Bernthal, Motley, Schwind and Weeks).

Though the assumption that the reactions are generalized would scarcely be questioned, it by no means follows that various portions of the peripheral vasculature participate in similar degree or even in accordance with the same qualitative pattern. Differences in the properties of neural units controlling vasomotor outflow to individual peripheral regions and differences in the access of chemoreceptor influence to these units, lack of uniformity in the richness of the efferent vasomotor innervation of various tissues, the presence or absence of sympathetic vasodilator fibers, and variations in the relative proportions of coexistent vasoconstrictor and vasodilator innervation are potentialities which invite anticipation of qualitative as well as quantitative dissimilarities in chemoreflex reactions in the several body regions.

Though data are plentiful concerning the general reactions as they are reflected in the arterial blood pressure, the contributions of individual parts of the vasculature in chemoreflex reactions have not been so extensively studied. Responses in the spleen (Heymans, Bouckaert, v. Euler and Dautrebande, 1932), in the limb (Bernthal, 1932, 1938; Heymans, Bouckaert and Handovsky, 1935) and in the submaxillary gland (Bernthal, Motley, Schwind and Weeks) have been described. Bearing in mind that the splanchnic region, and particularly the intestine, has long been considered especially important in generalized vascular reactions having to do with redistribution of circulating blood, we have performed experiments dealing with chemoreflex responses in the region supplied by the superior mesenteric artery. We have endeavored, first, to demonstrate the reactions and their general pattern and, second, to compare simultaneously occurring responses in the intestine and in the leg.

**METHODS AND PROCEDURE.** The index of vasomotor activity in these experi-

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ments was change of arterial blood volume flow under conditions of controlled driving head of blood pressure. Details of the method for supplying blood under constant conditions to the vascular beds concerned may be found in an earlier report (Bernthal, 1938). Briefly, a specially designed "perfusion" apparatus receives normally aerated blood from the aorta of the experimental animal and, without altering its chemical state or physical properties, delivers it to the arteries in which flow is being measured at an evenly pulsating pressure having a constant mean value. In prior use of this method (Bernthal, 1938; Bernthal and Weeks, 1939) the collateral arterial circulation of the region under study was interrupted as completely as possible to guard against artefacts which might arise from changing relationships between systemic arterial and perfusion pressures. In the present experiments, this procedure, though applicable to the leg, was thought to entail too great risk of mechanical abuse when applied to the intestine. However, the same end was sought by regulating the systemic mean arterial blood pressure as well as the mean perfusion pressure in such a way that the two were identical or in a constant ratio to each other. A circulating systemic blood pressure compensator similar to that described by Winder (1938) was attached to the aortic blood supply line of the perfusion equipment. The efficiency of this method can be gauged by inspection of the records reproduced in figures 1 and 2.

Blood volume flow was measured by means of the thermoelectric method of Gesell and Bronk (1926)<sup>3</sup>, the output of the thermopiles being automatically inscribed upon smoked paper by General Electric Photoelectric Recorders. In cannulating the superior mesenteric artery, special precautions were exercised to avoid injury to the intestine and its nerve supply. Cannulation and the completion of connections with blood flow recorder and perfusion equipment entailed interruption of the intestinal blood supply for approximately ten minutes.

Chemoreflex reactions were elicited by two different general procedures. In some experiments chemical changes were produced in the arterial blood by alterations of the composition of the inspired air or by intravenous injection of chemical

<sup>3</sup> Because of similarity in the underlying principle of this method and the thermistor method of Baldes and Herrick (1937), the accuracy of which has been challenged upon several counts (Gregg et al., 1942), it seems advisable to mention certain specific points in connection with our blood flow determinations. The Gesell and Bronk method is not subject to inaccuracy due to different or changing properties of various arteries in which flow may be measured because the artery itself is not involved in the heat exchange. Errors due to variations in environment were routinely guarded against by sheathing the units in cork and using them suspended in air and covered with a thick layer of dry cotton wool. The units never came into external contact with body tissues or tissue fluids. The potentiality of error due to variations in cyclic flow pattern became apparent early in our use of the thermoelectric method. Such variations, especially backflow, were minimized by the use, whenever possible, of rigid conduits in all parts of the perfusion circuit proximal to the units and also as far distally as possible, and by the use of a standard uniform pulsation rate (100/minute) in all experiments and calibration procedures. *In vitro* calibrations with heparinized dogs' blood were carried out with the units mounted on the perfusion equipment exactly as they were used in animal experiments. The viscosity of the blood was not controlled and presumably it varied in the experiments. Partly for this reason, direct comparisons of flow values made in this report pertain to points in the experiments separated by time intervals as short as possible.

agents. In these experiments carotid and aortic chemoreceptor influences could be eliminated separately or together by means of silver cold blocks applied to Hering's nerves and to the cervical (vago-sympathetic) aortic nerve trunks. In other experiments chemical changes were confined to the carotid bodies alone by a technique described earlier (Bernthal, 1938). In neither procedure were direct peripheral effects of the chemical stimuli involved in the reactions, because the large combined capacity of the perfusion equipment and pressure compensator made it possible to supply normal arterial blood to the axillary and superior mesenteric arteries during each application of stimulus and throughout the resulting reflex reaction and subsequent recovery. The reactions shown are therefore predominantly the result of nervous control.

Eight experiments were performed upon heparinized dogs (9-12 kgm.) narcotized with morphine (7 mgm. per kgm.) and urethane (1 gram/kgm.). Heparinized or defibrinated blood from a donor animal was used for initial filling of the systemic arterial blood pressure compensator and perfusion apparatus.

**RESULTS AND DISCUSSION.** *The responses in the intestinal vasculature.* The initial and basic response of the intestinal vasculature to increased chemoreceptor activity is constriction, in which respect this extensive portion of the vascular bed behaves as do vessels of the spleen, the limb and the submaxillary gland (see references cited earlier). This common response is to be expected upon the basis that carotid and aortic chemoreceptor stimulation initiates generalized excitation of thoracolumbar vasomotor efferents.

The records shown in figure 1 are all taken from the same experiment and are typical in their pattern. Inhalation of 1 per cent oxygen in nitrogen resulted in vasoconstriction sufficient to lessen the blood volume flow in the superior mesenteric artery by 19 per cent (fig. 1A). Close interdependence and reciprocity between respiratory and vasomotor responses are demonstrated by comparison of figures 1A and 1B, for substitution of constant artificial pulmonary ventilation for the naturally controlled hyperpnea of figure 1A intensified the hypoxic vasoconstriction sufficiently to reduce the blood flow by 65 per cent.

The relative contributions of aortic and carotid chemoreceptors to the reaction are indicated by figures 1C and 1D. Just preceding 1C both Hering's nerves were cold blocked, leaving the pathways from the aortic chemoreceptors intact. The hypoxia then produced only a 9 per cent reduction in blood flow (fig. 1C). On the other hand, with the carotid chemoreceptor pathways intact and those from the aortic bodies blocked (fig. 1D) the blood flow was reduced by 31 per cent. In some of the animals this relationship was the converse.

That the responses shown were truly chemoreflex was shown by the effects of simultaneous cold blocking of Hering's nerves and the cervical (vago-sympathetic) aortic nerve trunks, whereupon there remained no recognizable vasoconstrictor response either to hypoxia (fig. 1E) or to cyanidemia (figs. 1F and 1G) of the degree employed in these experiments.

*Responses in intestine and leg compared.* Though accumulated evidence indicates that chemoreflex vasomotor responses are basically similar in several body regions, comparative observation of simultaneously transpiring components of

such responses may, nevertheless, be informative. It is axiomatic that, so far as redistribution of circulating blood is concerned, the effectiveness of any generalized vascular response must depend wholly upon differences in its pattern and in its intensity in the several body regions. Further, comparison of the simultaneous responses of effectors activated reflexly from a common source must suggest properties of intervening portions of the control mechanism.

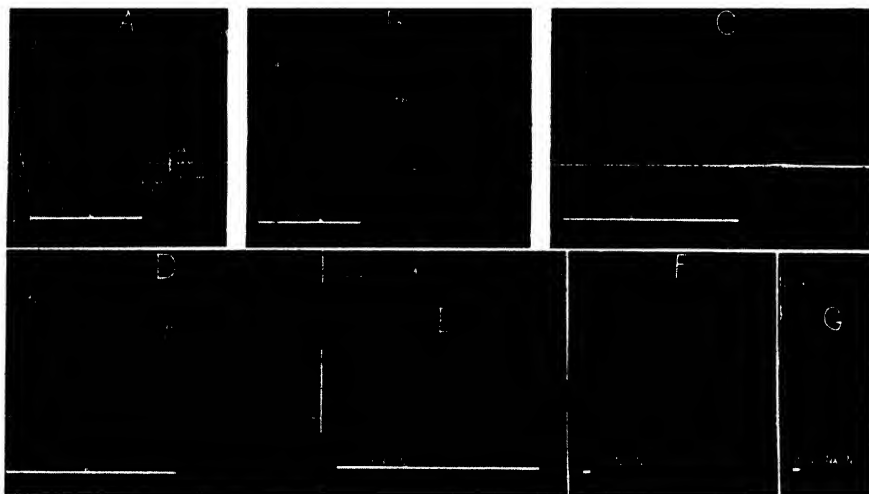


Fig. 1. Chemoreflex vasomotor reactions in the intestine.

*SM*, volume flow of blood in superior mesenteric artery in cc./min. indicated by numerals adjacent to curves. *R*, spirometer record. *BP*, artificially regulated systemic arterial blood pressure, mercury manometer; zero on signal line. *PP*, pressure of blood supply to superior mesenteric artery; zero on time line. *S*, signal. *T*, time in intervals of 1 and 10 seconds.

A. Effects of inhalation of 1 per cent oxygen. Hering's nerves and aortic nerves intact. Natural breathing.

B. Repeat 1 per cent oxygen inhalation during constant artificial pulmonary ventilation with open pneumothorax.

C. Repeat hypoxia. Hering's nerves blocked; aortic nerves intact.

D. Repeat hypoxia. (Vago-sympathetic) aortic nerves blocked; Hering's nerves intact.

E. Repeat hypoxia. Hering's nerves blocked; (vago-sympathetic) aortic nerves blocked.

F. Effects of sodium cyanide, intravenous administration. Hering's nerves and aortic nerves intact.

G. Repeat cyanide after cutting Hering's nerves and the (vago-sympathetic) aortic nerves.

In figure 2 are shown simultaneous recordings of chemoreflex changes of blood flow in the axillary and superior mesenteric arteries and they demonstrate that the *pattern* of response is not identical in the two vascular beds. One of the differences is to be seen only during more extended applications of stimulus as in figures 2B and 2C. In reactions such as these, the vessels of the extremity characteristically display their fullest intensity of response early, after which the constriction may either remain steady or recede in part. The vessels of the

intestine, on the other hand, often exhibit a more gradually intensifying constriction which does not reach its full strength until considerably later in the reaction. Consequently, as figures 2B and 2C demonstrate, during the later phases of such responses, the vascular reactions in the two regions may proceed in different directions even though they are simultaneously under the control

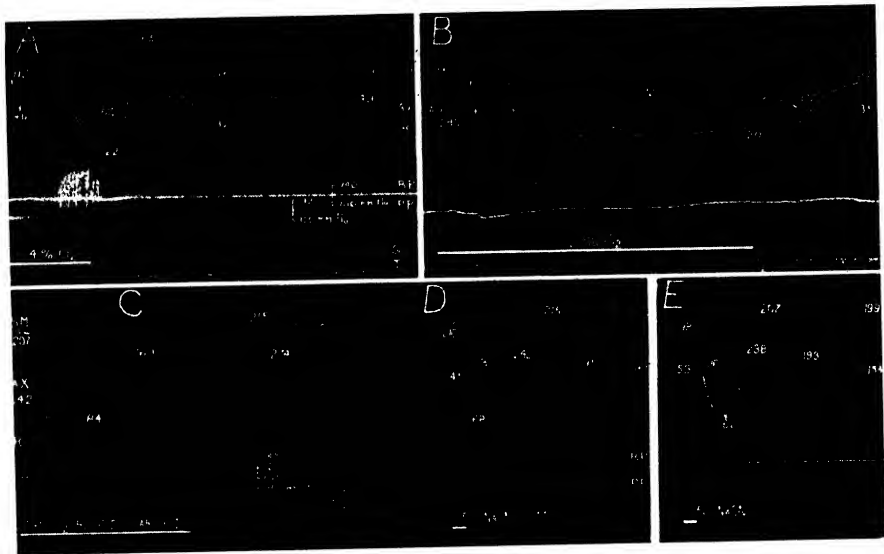


Fig. 2. Simultaneous chemoreflex vasomotor reactions in foreleg and intestine.

AX, volume flow of blood in right axillary artery. All other abbreviations as in figure 1. Both axillary and superior mesenteric arteries supplied with blood at constant mean pressure indicated by PP.

A. Effects of hypoxia. All chemoreceptor pathways intact. Natural breathing. Note absence of post-hypoxic increased blood flow in the intestine.

B. Prolonged hypoxia. Description in text. Vagi cut. (Arterial blood pressure compensator not operating.)

C. Localized hypoxia at carotid chemoreceptors alone. Perfusion of vascularly isolated carotid bifurcations with blood previously taken from aorta of experimental animal during inhalation of 2+ per cent oxygen.

D. Five-tenths cubic centimeter of 0.01 M NaCN injected into blood supply of vascularly isolated carotid bifurcations.

E. Same. Natural breathing.

of a common afferent stimulation. Whether this partial independence in response springs from characteristics of the control mechanism or of the effector mechanism cannot be stated upon the basis of existing information.

The most striking and constant difference in pattern of response in the two regions appears in the interval immediately following the termination of hypoxia or cyanidemia while the environment of the chemoreceptors presumably is returning to normal and during which the chemoreceptors are believed to experience a silent period or a diminution of their rate of discharge below the preadministration level of tonic activity (Bernthal, 1938). During this period the



leg vessels undergo a very marked and abrupt dilatation (e.g., sufficient to allow blood flow to increase 258 per cent above preadministration values in fig. 2A), whereas in the intestine the vessels simply return steadily toward their normal level of tone either with no dilatation at all (fig. 2A) or with a very small dilatation above the prehypoxic level (figs. 2C, D and E). That post-hypoxic dilatation in the leg is not dependent upon purely local phenomena (e.g., reactive hyperemia) but reflects changing activity of the central nervous controlling mechanism is indicated by the occurrence of corresponding changes in breathing movements (figs. 2A and E) and in heart rate. Though hypocapnia of the vasoconstrictor center following the period of pulmonary hyperventilation is no doubt a contributing factor, this cannot be the basic cause since the phenomenon persisted even when constant artificial pulmonary ventilation was imposed (see figs. 2C and D). The obvious probability is that the post-hypoxic vasodilatation coincides with temporary hypoactivity of the vasoconstrictor center, such hypoactivity resulting from abrupt cessation of a markedly increased stream of excitatory chemoreceptor impulses. Pitts, Larrabee and Bronk (1941) have, in fact, strikingly demonstrated such a temporary hypoactivity in spontaneously active sympathetic fibers (presumably vasomotor) immediately following intense stimulation of vasoconstrictor neurones from an extraneous source (the hypothalamus). The latter demonstration, furthermore, is in opposition to an alternative hypothesis that the post-hypoxic vasodilatation represents after action upon the part of sympathetic vasodilator fibers, which Bulbring and Burn (see Burn, 1938) believe to be relatively numerous in the leg of the dog as compared with the intestine.

The explanation for the absence of post-hypoxic vasodilatation in the chemoreflex response of the intestinal vasculature is not clear, but the possibilities which it suggests may be significant. Granting that post-hypoxic vasodilatation in the leg does indicate a recession of the activity of the vasoconstrictor center below its normal level of tonic discharge, then smallness or absence of a parallel phenomenon in the intestine suggests either that normal arteriolar tone in that region is relatively independent of the tonic activity of the center or that there are compensating mechanisms in the intestine capable of locally opposing such vasodilatation. While conclusions upon so important a point may properly await more direct evidence, it is pertinent to recall the findings of H. W. Smith et al. (1939) which led them to conclude that (in unanesthetized man) "the tone of the renal arterioles is normally maintained by autonomous intrinsic activity of the peripheral vascular apparatus and is not dependent upon tonic activity of the central nervous system" and to infer that the same is true of the arterioles of the other splanchnic viscera.

The possibility of a local reflex vasoconstrictor influence capable of opposing post-hypoxic vasodilatation in the intestine is suggested by the findings of Gammon and Bronk (1935) that elevation of pressure within the intrinsic vessels of the Pacinian corpuscles of the mesentery evoked increased discharge from these structures. Their experiments suggested, further, that such increased discharge reflexly induced constriction in the intestinal vessels. The onset of a vasodilata-

tion which included the arterioles of the Pacinian corpuscles (and thus raised the pressure within their intrinsic vessels) might conceivably initiate a self-limiting local compensatory reaction.

Interest attaches to the *intensity* of the vasomotor reactions occurring in the intestine in comparison with those occurring elsewhere. It is well known that in hypoxia, and in certain other situations which involve the chemoreflex mechanism, vasomotor reactions are brought into play which effect a redistribution of arterial blood flow in favor of brain and myocardium. The contribution of the splanchnic region relative to that of others such as the extremities has never been determined quantitatively, although the former is often emphasized in discussions.

In these experiments comparisons have been made upon the basis of changes in blood volume flow which occurred simultaneously in leg and intestine. Inasmuch as the perfusion pressure was a constant under the experimental conditions, the following relationship was assumed,

$$\frac{\text{blood volume flow before reaction}}{\text{blood volume flow during reaction}} = \frac{\text{peripheral resistance during reaction}}{\text{peripheral resistance before reaction}}$$

Upon this assumption, the values for the ratio  $VF_1/VF_2$  in table 1 indicate intensity of vasomotor response in terms of multiples of whatever peripheral resistance to flow existed in the respective vascular beds just preceding the response. Further, since identical constant perfusion pressures were used for the two regions, the intensity of reaction in the intestine as compared with the simultaneous reaction in the leg was expressed by the value of the ratio,  $\Delta PR\text{-sup. mes.}/\Delta PR\text{-ax.}$  (last column, table 1).<sup>4</sup>

The results presented in table 1 comprise a representative series taken from several different experiments. The figures demonstrate wide variations in the relative intensity of response in the two regions. Sometimes (one experiment) the reactions in the intestine were consistently the more intense. Sometimes the relative intensities became reversed at different stages of the same experiment. In by far the greater number of instances, however, the vasomotor responses in the intestine were consistently definitely less pronounced than those in the leg, i.e., in the generalized vascular reaction resulting from chemoreceptor stimulation, the peripheral resistance offered by the intestinal vasculature was increased in lesser degree than was the peripheral resistance offered by the leg vessels. It follows, at least when the chemoreflex factor dominates the vascular response as in hypoxia, that if the intestinal vascular bed does actually exert a greater influence than that of the leg and similar regions in the process of redistribution of blood volume flow, this superiority must usually depend upon factors other

<sup>4</sup> Figures for  $\Delta PR\text{-sup. mes.}/\Delta PR\text{-ax.}$  indicate relative intensity of maximum phase of responses in intestine and leg, no attempt having been made to estimate averages of fluctuating flow rates throughout entire reactions. An important corollary question, not considered here, is the relative durability of response in leg and intestine during prolonged chemoreceptor stimulation.

TABLE 1

EXPERIMENT	PROCEDURE	DESCRIPTION	PERFUSION PRESSURE  <i>mm Hg</i>	VOLUME FLOW OF BLOOD (CC.MIN.)				VF <sub>1</sub> /VF <sub>2</sub>		$\Delta$ PR-SUP. MES./ $\Delta$ PR-AX.
				Prestimulation mean (VF <sub>1</sub> )		Stimulation Max. response (VF <sub>2</sub> )				
				Superior mesenteric	Axillary	Superior mesenteric	Axillary	Superior mesenteric	Axillary	
2	1	5% O <sub>2</sub> trachea. All chemoreceptors*	120	166	45	152	38	1.09	1.18	0.50
	2	Repeat 1	120	166	43	150	37	1.10	1.16	0.62
	9	5.0 cc. 0.01 M NaCN-vein. Vago-aortic nerves cut*	120	138	47	86	25	1.60	1.88	0.70
	12	2.0 cc. NaCN-vein. Vago-aortic nerves cut*	120	166	38	146	22	1.13	1.72	0.18
	20	2.0 cc. NaCN-vein. Vago-aortic nerves cut†	120	195	47	169	29	1.15	1.62	0.24
	30	Same as 20	120	183	63	167	38	1.09	1.66	0.14
3	1	10% CO <sub>2</sub> trachea. All chemoreceptors*	116	116	48	93	39	1.25	1.23	1.09
	3	3-4% O <sub>2</sub> trachea. All chemoreceptors*	116	112	46	62	22	1.80	2.09	0.73
	7	3-4% O <sub>2</sub> trachea. Hering's nerves blocked*	116	107	37	77	20	1.39	1.85	0.46
	15	Same as 7	116	90	46	68	30	1.32	1.53	0.60
	22	3-4% O <sub>2</sub> trachea. All chemoreceptors†	98	79	41	46	25	1.72	1.64	1.12
		Terminal asphyxia	96	78		14—		5.60		
4	3	7% O <sub>2</sub> trachea. All chemoreceptors*	92	162	49	146	37	1.11	1.32	0.34
	8	Same†	92	136	49	113	39	1.20	1.26	0.77
	25	100% N <sub>2</sub> trachea. Vago-aortic nerves cut†	94	119	54	98	35	1.21	1.54	0.39
	37	Same as 25	94	154	39	125	29	1.23	1.34	0.67
	38	5.0 cc. 0.01 M NaCN-vein. Vago-aortic nerves cut†	94	132	37	81	19	1.62	1.95	0.65
6	1	3% O <sub>2</sub> trachea. Carotid chemoreceptors excluded*	72	25	72	11	47	2.27	1.51	2.49
	3	3% O <sub>2</sub> trachea. All chemoreceptors*	72	22	86	11	56	2.00	1.53	1.88
	6	3% O <sub>2</sub> trachea. Vago-aortic nerves cut*	72	21	113	15	84	1.40	1.34	1.18
		Terminal asphyxia		107		8		13.33		
8	4	2% O <sub>2</sub> trachea. Carotid chemoreceptors excluded*	98	150	94	45	44	3.33	2.14	2.04
	23	2% O <sub>2</sub> trachea. All chemoreceptors* Vagi cut	85	200	122	100	90	2.00	1.35	2.86
	52	2+% O <sub>2</sub> blood-isolated carotid chemoreceptors†	88	203	142	169	84	1.20	1.69	0.29
	50	0.5 cc. .01 M NaCN-isolated carotid chemoreceptors*	88	198	153	116	62	1.71	2.47	0.48
	54	0.5 cc. NaCN-isolated carotid chemoreceptors†	88	209	145	131	68	1.59	2.13	0.52

\* Natural breathing.

† Uniform artificial pulmonary ventilation.

PR (last column) = peripheral resistance.

than the intensity of its constrictor response, e.g., upon its area or the magnitude of its normal blood volume flow.

These experiments are not alone in suggesting that the reaction of the intestinal arterioles in compensatory vasomotor adjustments may be moderate. In this connection it is pertinent to recall the experiments of Herrick, Grindlay, Baldes and Mann (1940) concerning the effects of muscular exercise and those of Rein and Rossler (1929) concerning vascular adjustments to hemorrhage. The former investigators found that while the vessels of the intestine in their animals may have constricted during exercise, the constriction was not sufficient to decrease the volume flow of blood in the superior mesenteric artery in the face of rising arterial blood pressures. Rein and Rossler found, in those of their experiments which were performed at ordinary environmental temperatures, that hemorrhage resulted usually in a much smaller percentage reduction in blood volume flow in the intestine than in the extremities.

That the relatively moderate participation of the intestinal arterioles in many of the chemoreflex responses was not dependent upon incapacity for more intense response, either on the part of the vessels themselves or of the central control mechanism, became apparent in a number of ways. For example, in an experiment (no. 4, table 1) in which nitrogen inhalation (carotid chemoreceptors alone) produced a value of 1.23 for the ratio in the intestine of *blood volume flow before reaction/blood volume flow during reaction*, the reflex vasoconstriction resulting from localized stretching of a small segment (1 cm.) of ileum between forceps was sufficient to make this ratio value 2.0. More striking, during mechanical asphyxia used to dispatch an animal at the end of an experiment (no. 3, table 1) vasoconstriction in the intestine was sufficiently intense to produce a value of 5.6 for the ratio, whereas the value for the most intense chemoreflex reaction during the experiment proper was only 1.8 (3-4 per cent oxygen inhalation with all chemoreceptors intact). Again (expt. 6, table 1) the ratio value for 3 per cent oxygen inhalation was 2.27, but the value for the terminal asphyxiation was 13.3 (flow in superior mesenteric artery decreased from 107 to 8 cc./min., perfusion pressure remaining constant, systemic arterial pressure falling because of cardiac failure). Thus the potential capacity for vasomotor response upon the part of the intestinal vessels and their nervous controlling mechanism is shown to be very great but the intensity of stimulation required to implement this capacity fully appears to be so high that it is brought into action only under most extreme conditions.

The phenomenon of a shifting or reversing relative intensity of response of intestinal and limb vasculature in individual animals (last column, table 1) excites curiosity as to its mechanism, the more so since it probably has to do with properties of the nervous mechanism for vasomotor control, alterations of local chemical and hormonal influences in the two regions concerned having been minimized by the experimental conditions (perfusion of limb and intestine with normal arterial blood, thus excluding there the chemical changes brought to bear upon chemoreceptors and central nervous system). Rein and Rossler (1929) were able to reverse the relative intensities of the blood flow changes in gut and

in the intestine tending to be relatively more intense during stronger chemoreceptor stimulation and relatively less intense during weaker chemoreceptor stimulation under otherwise comparable conditions.

Under extreme conditions involving combined intense centrogenic and chemoreflex stimulation (prolonged asphyxiation) the intestinal vascular bed was shown to possess a capacity for constriction several times greater than any exhibited in response to intense chemoreceptor stimulation alone.

The data reflect that aspect of vasomotor response having to do with redistribution of blood volume flow and do not inform in any quantitative sense concerning chemoreflex changes in capacity of the vascular system or of its parts.

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# EFFECTS OF TEMPERATURE CHANGE ON THE WATER BALANCE IN MAN

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Maintenance of a constant body temperature in a changing environment is effected in part by the variation in the exchange of heat between the body and its surroundings. This transfer of heat is determined chiefly by conduction, convection, and radiation and by the evaporation of water from the body surface. These processes, which depend on the temperature of the skin, are controlled by alterations in the peripheral circulation. Thus the distribution and exchange of the water of the body are influenced by variations in the temperature of the environment.

Besides the more obvious changes in water metabolism (e.g., increased fluid intake, sweating, etc.) which occur on exposure to heat, evidence has been obtained that fluid shifts within the body may possibly play a part in the temperature regulation. Bazett et al. (1940) have found that the circulating blood volume increases significantly on prolonged exposure to high temperatures. Such an increase of the blood volume would help to account for the increase in the peripheral circulation which occurs during exposure to heat. Evidence has also been advanced to show that alterations in the sweat mechanism appear on acclimatization to high temperatures (Dill et al., 1933; Winslow et al., 1938; Burton et al., 1940). Furthermore, it is recognized that the incidence and severity of edema are greater during hot weather than during cold weather. It is evident, therefore, that changes in the temperature of the external environment will be accompanied by the movement of water within the body.

The purpose of this study was to observe the changes which take place in the fluid balance of normal human subjects when an alteration in the environmental temperature occurs, all other conditions being controlled as carefully as possible. The general plan of all the experiments was essentially the same; in each, two subjects were kept under constant observation for a period of about one week. During approximately one-half of this time the temperature was maintained at a relatively low level, and in the other half of the period the temperature was high.

**PROCEDURE.** Three experiments were performed on six normal, white, adult males, who lived in the vicinity of New York City and were engaged in sedentary work in the Medical School. Since the subjects were trained in investigative work, they were able to co-operate in making many of the observations. Physical examination showed them to be in good health and without apparent physical defects.

Experiment 1 was a preliminary study in which the precision of control of the environmental temperature was somewhat less than in the subsequent experiments. The warm period, August 2 to August 9, 1940, was spent by subjects A and B in normal summer weather. The mean temperatures on August 8, 9, and 10 were, respectively, 73.6°, 76.2°, and 75.5°F. On August 8 the control diet was initiated, and two days later the subjects entered the constant temperature room where a dry bulb temperature of 60.5°F.  $\pm 2.0^\circ\text{F}$ . and a wet bulb temperature of 44.5°F.  $\pm 1.0^\circ\text{F}$ . was maintained. Except for a twenty minute period each day, the subjects remained in this room until August 15.

Experiment 2 with subjects C and D consisted also of a warm period followed by cold, and was performed during the week of December 17 to December 24, 1940. The weather during the preceding week was cold with average maxima and minima of 45°F. and 33°F. Controlled diet was started on December 17, and the subjects entered the constant temperature room on that afternoon. The room was maintained at 80.2°F.  $\pm 0.7^\circ\text{F}$ . D.B., and 63.5°F.  $\pm 0.5^\circ\text{F}$ . W.B., until December 21 at which time the conditions were changed to 60.0°F.  $\pm 0.7^\circ\text{F}$ . D.B., and 47.5°F.  $\pm 0.5^\circ\text{F}$ . W.B. It required about five hours for the system to reach this new level which was held until the subjects were discharged on December 24. At no time during the experiment did the subjects leave the conditioned room.

Experiment 3 with subjects E and F extended from December 17 to December 24, 1941, and differed from experiments 1 and 2 in that the temperature change occurred in the reverse order from the previous experiments, namely, from cold to warm. The weather temperatures during the preceding week had average maxima and minima of 41°F. and 30°F. Controlled diet was started on December 17 and the subjects entered the room on that evening. Temperatures were maintained at 64.3°F.  $\pm 0.4^\circ\text{F}$ . D.B., and 47.7°F.  $\pm 0.5^\circ\text{F}$ . W.B., until December 21 and on that day changed to 86.8°F.  $\pm 0.6^\circ\text{F}$ . D.B., and 71.5°F.  $\pm 0.5^\circ\text{F}$ . W.B., and held for the remainder of the experiment. As in experiment 2, the subjects at no time left the conditioned room. The average air movement in all three experiments was maintained at 14 feet per minute over the center of the chamber. In all experiments the subjects were permitted to select clothing suitable to each environmental condition.

The experiments were performed in an air-conditioned, windowless chamber designed for maintenance of uniform temperature, humidity and air movement throughout the room. The thickness of the insulation prevented fluctuations in wall temperature; this was of utmost importance since it ensured constant conditions of radiant heat exchange. Observers were able to enter and leave the experimental room through an insulated door without affecting the conditions in the room. Continuous recordings of wet and dry bulb temperatures were made by means of an aspirating psychrometer employing resistance thermometers sensitive to 0.1°F. A hot wire anemometer constructed by Dr. A. P. Gagge was used to measure the air movement. The chamber was equipped with a two-way speaker system for communication with an adjacent laboratory. Electrical measuring instruments in the chamber were wired through conduits to recording devices outside of the experimental room.

Surface temperatures of the subjects in experiments 2 and 3 were measured at fifteen points by means of a radiation thermopile using the technique described by Hardy (1934); the mean values for the entire body surface were computed according to the method employed by Winslow et al. (1936). Skin temperatures beneath the clothing were measured by means of a thermocouple. Rectal temperatures were determined by a rectal thermometer and occasionally by a thermocouple and potentiometer. Temperature measurements were made in duplicate in the late afternoon.

The diets were prepared by the Metabolism Service of the Presbyterian Hospital. Food for each experiment was purchased at one time in order to ensure uniformity, and all inedible portions of the food (i.e., bones, fruit rinds, etc.) were removed. Each item of the diet was carefully weighed before cooking and the diet for each day was identical in weight and composition. The food was selected and prepared in order to have a low sodium chloride content (i.e., salt-free bread and butter). To this diet the subjects added a constant and accurately weighed quantity of sodium chloride, thereby making day-to-day variations in the salt intake negligible. The subjects were required to eat all their food and to mop their dishes with dry bread. Loss of water from the food during

TABLE 1

EXP. NO.	CALORIES	CARBO- HYDRATE	PROT.	FAT	Na	Cl	WATER	SALT ADDED	TOTAL SOLIDS
		<i>gms.</i>	<i>gms.</i>	<i>gms.</i>	<i>gms.</i>	<i>gms.</i>	<i>gms.</i>	<i>gms.</i>	<i>gms.</i>
1	2125	217	91	99	0.99	1.20	1643	10	420
2	2453	270	98	109	1.10	1.50	1660	8	480
3	2502	235	90	133	0.93	1.27	1550	8	472

cooking was calculated by reweighing the food immediately before it was eaten. Water was allowed ad libitum, but the quantity consumed was accurately measured. The water produced by oxidation of the food was calculated and added to the water intake. Table 1 shows the composition of the diet provided in each of the three experiments.

The experimental data were obtained on the basis of twenty-four hour periods, each period commencing at 8 a.m. Each morning at seven o'clock, before the subjects arose, blood samples were drawn without stasis. These blood samples and also the daily accumulations of urine were analyzed for chloride by the method of Volhard (1878) and for sodium by the method of Butler and Tuthill (1931), the analyses being made in duplicate. Hematocrit values were determined daily on heparinized blood in Wintrobe tubes centrifuged at 3000 r.p.m. for forty-five minutes. Simultaneous determinations of plasma volume and "available fluid" (NaSCN) were made according to the method of Gregersen and Stewart (1939). The total blood volume (BV) was calculated from the plasma volume (PV) and the hematocrit reading (HCT) by using the relationship:  $BV = 100 PV / (100 - HCT)$ . Insensible weight loss was measured by a Buffalo beam balance sensitive to two grams. Weighings were made at half-hour intervals, and the weight loss computed for different periods of the day. The



extrarenal water loss was estimated by the procedure of Newburgh et al. (1937). This consists of correcting the daily insensible weight loss for change in weight due to the respiratory gas exchange, the amount of correction being computed from the normal metabolism of the diet. Feces were weighed for each twenty-four hour period, but were not subjected to analysis. The basal metabolic rate was measured in experiment 2 by the Jones metabolism apparatus. This observation was made each morning before the subjects arose, and the recorded values are the mean of two or three tests.

**RESULTS.** *Water, sodium and chloride.* The details of the water balance for all subjects are shown in figure 1. The data on these charts were computed on a twenty-four hour basis beginning at 8:00 in the morning of the day indicated. The caloric requirements for the experiments appear to have been underestimated, since all subjects showed a gradual loss of weight. No trend of weight change with rise or fall of temperature was observed. The most striking feature of the results summarized in figure 1 is the constancy of the extrarenal water loss when the environmental temperature is held constant. This means that the activity of the subjects was successfully maintained at a constant level throughout the experiments.

The insensible weight losses, given in table 2, show no significant differences between morning, afternoon, and evening rates. However, at night during sleep the insensible weight loss was appreciably lower; for one subject (E) during the cold period the average value for several nights was sixteen grams per square meter per hour.

The serum sodium and the serum chloride values which are given in tables 3 to 8 show no significant variations with temperature.

The daily renal excretion of sodium and chloride (fig. 1) was less during the warm periods than during the cold periods, the difference being attributed to the loss in the sweat. The subjects in experiment 1 showed large renal excretions of both sodium and chloride on the first day of exposure to cold after the warm period. In experiment 2 these effects are less marked. In experiment 3, where the temperature change is reversed, i.e., from cold to warm, there is no suggestion of a corresponding retention of sodium or chloride. Since no measurements were made of the salt lost through the skin or in the feces, it was not possible to compute accurately the relative changes in the volume of fluid available for distribution of sodium and chloride.

*Plasma volume determinations.* A graphic view of the plasma volume changes is given in figure 2. In each experiment the plasma volume was measured once during each constant temperature period. The plasma volumes for the other days were calculated from the hematocrit determinations. Both computations were employed for the last day of each experiment, and the agreement between them indicates the validity of the interpolated values. Experiment 1 was performed in the summer, and on exposure to cold both subjects showed a prompt rise in the hematocrit reading, suggesting a decreased plasma volume. However, three days later, when the plasma volume was again measured, the value for subject A was only 8 per cent below the control level and the value for B only

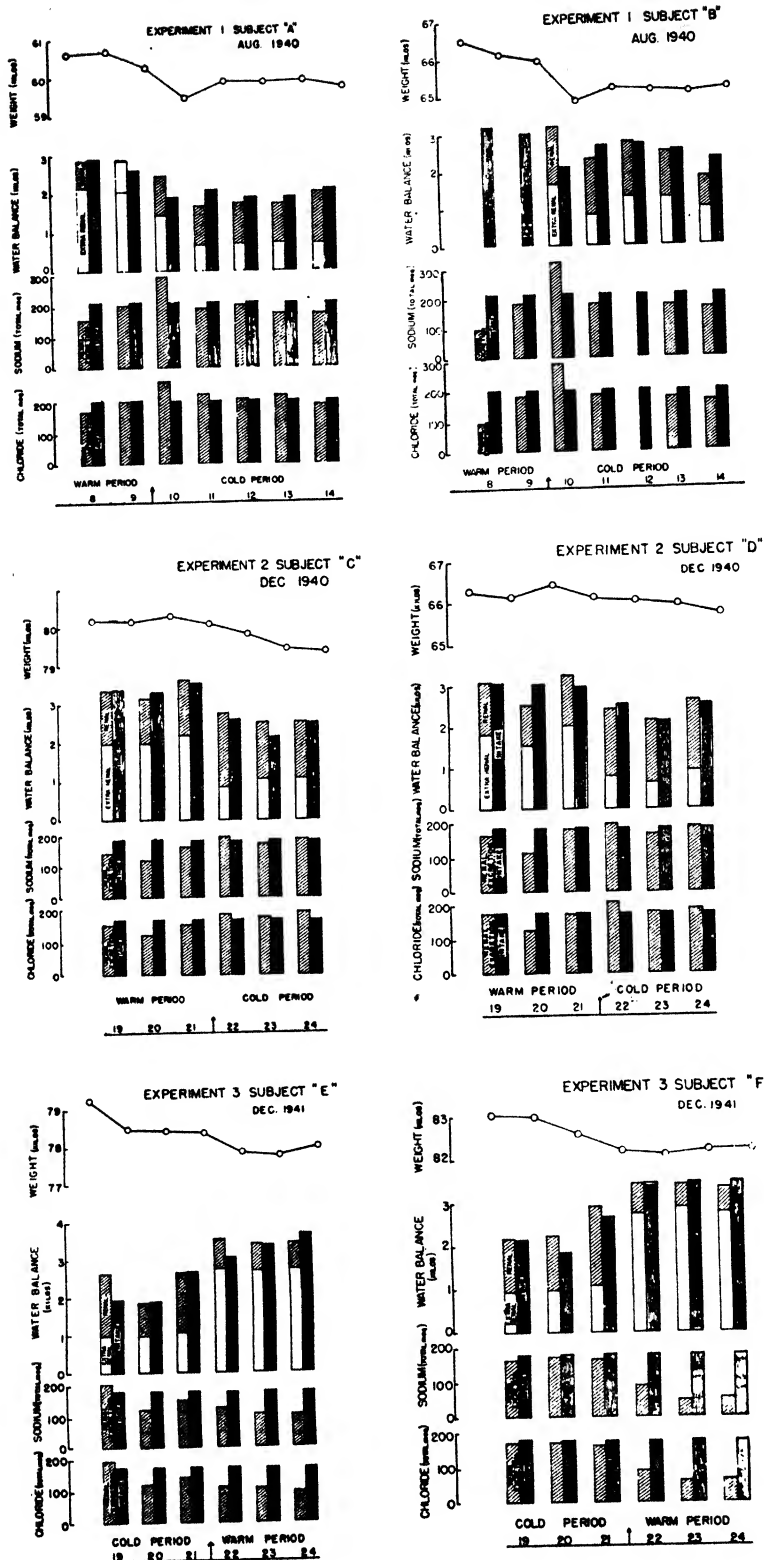


Fig. 1. Showing the daily weight change, water intake, renal and extrarenal water loss, and the intake and renal excretion of sodium and chloride. The daily weight changes and the water balance are plotted on the same scale to permit direct comparison.

7 per cent below this level. These results suggest that exposure to cold is associated with a temporary reduction in plasma volume followed by a gradual return toward the control over a period of a few days.

TABLE 2

*Insensible weight losses*

The numbers are the average weight loss in grams per square meter per hour for the corresponding hours of three days during each environmental temperature condition.

SUBJECT	ENVIRONMENT	MORNING	AFTERNOON	EVENING	OVERNIGHT
A	warm	49	88	30	34
	cold	24	25	22	18
B	warm	33	27		
	cold	31	24	31	22
C	warm	49	47	48	36
	cold	26	24	26	22
D	warm	37	47	39	32
	cold	30	21	23	18
E	cold	21	23	20	16
	warm	55	64	63	44
F	cold	21	23	26	20
	warm	62	68	84	40

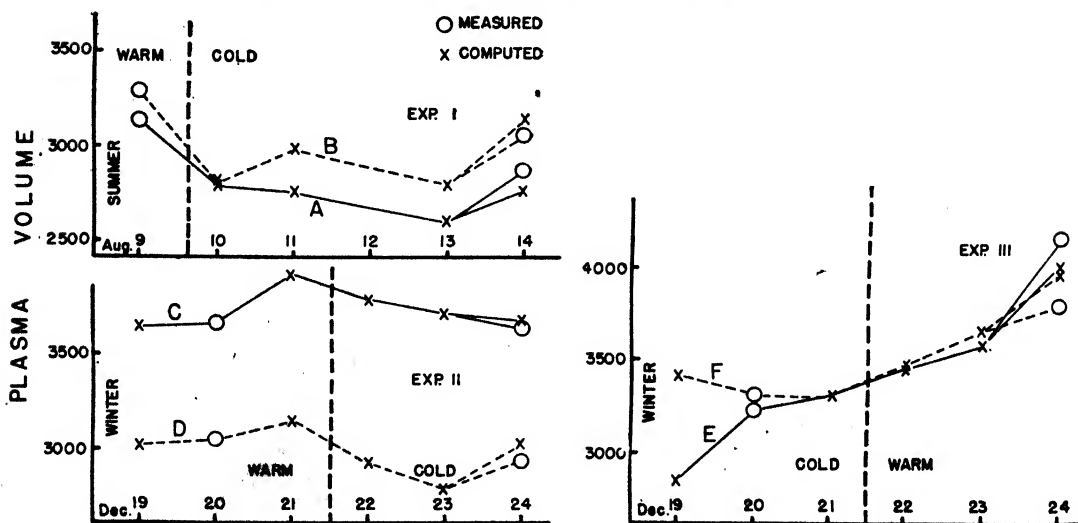


Fig. 2. Illustrating the plasma volume changes occurring with variations in the environmental temperature. The circles represent the plasma volumes measured directly by T-1824; the crosses show the plasma volumes computed from the first dye measurement by using the hematocrit value and assuming a constant total cell volume. The comparison of the two values on the last day of each experiment indicates the validity of the interpolation.

Experiment 2 was performed in the winter. During the initial warm period both subjects showed a decreasing hematocrit indicative of an increasing plasma volume. On exposure to cold the hematocrit values increased suggesting a decreasing plasma volume. As in experiment 1 after several days' exposure to cold the plasma volume showed a tendency to return toward the control level. The plasma volumes measured after three days' exposure to the cold were for subjects C and D, respectively, 3 and 4 per cent below the control level.

The most marked plasma volume changes were observed in experiment 3. This experiment was performed in the winter and consisted of a cold period fol-

TABLE 3

Experiment 1, Subject A, male, 25 years, single, laboratory worker. Height 176 cm., Surface area 1.74 sq. m. Daily intake—sodium 214 meq., chloride 205 meq.

AUGUST 1940	WARM PERIOD		COLD PERIOD				
	8th	9th	10th	11th	12th	13th	14th
Morning nude weight 7th—60662 (grams).....	60684	60250	59453	59907	59880	59910	59735*
Water intake (cc.).....	2907	2607	1907	2107	1907	1907	2107*
Renal excretion of water (cc.).....	700	810	1045	1000	1030	990	1310*
Extrarenal water loss (cc.).....	2145	2050	1421	688	709	749	722*
Renal excretion of sodium (meq.).....	155	203	296	194	202	177	174*
Renal excretion of chloride (meq.).....	172	202	266	222	210	221	189*
Serum sodium (meq./liter).....		146	143	145		142	143†
Serum chloride (meq./liter).....		104	104	102		99	102†
Plasma protein (gm. %)...		7.00					7.20†
Hematocrit (%).....		46.0	48.8	49.1		50.6	49.0†
Plasma volume (cc.).....		3130					2880†
Blood volume (cc.).....		5790					5650†
Available fluid (NaSCN) (cc.).....		15050					14700†

\* For the 24 hour period ending at 8 a.m. on the date given.

† For a fasting state in the morning.

lowed by a warm period. At the end of the warm period both subjects showed large increases in plasma volume. For subject E the increase was 29 per cent and for subject F it was 15 per cent above the control value. The trend of the hematocrit values suggests that the plasma volumes were still increasing at the time of the conclusion of this study.

*Available fluid.* Determination of the volume of fluid available for the dilution of sodium thiocyanate was made once on each subject in each constant temperature period. These measurements are recorded in tables 3 to 8. Experiments 1 and 2, involving environmental changes from warm to cold, demonstrate for subjects A, B, C and D respectively reductions in the available fluid volume of 2, 1, 14 and 6 per cent. Experiment 3, in which the environment changes from cold

to warm, shows for subject E an increase in available fluid volume of  $2\frac{1}{2}$  per cent and for subject F no change at all. Since the experimental error in this method may be as large as 3 per cent, only the changes found for subjects C and D can be considered significant. From these measurements, therefore, there is no indication that the volume of the extracellular fluid compartment is regularly affected to any marked extent by variations in the environmental temperature.

*Temperature measurements.* Rectal temperatures remained relatively constant throughout all the experiments and showed no trend with changing environment. Skin and surface temperatures (tables 5 to 8) demonstrated striking differences

TABLE 4

Experiment 1, Subject B, male, 24 years, married, laboratory technician. Height 183 cm., Surface area 1.87 sq. m. Daily intake—sodium 214 meq., chloride 205 meq.

AUGUST 1940	WARM PERIOD		COLD PERIOD				
	8th	9th	10th	11th	12th	13th	14th
Morning nude weight 7th—66510 (grams).....	66150	65950	64870	65200	65120	65080	65180*
Water intake (cc.).....			2090	2650	2700	2510	2300*
Renal excretion of water (cc.).....			1555	1490	1480	1230	835*
Extrarenal water loss (cc.)..			1630	820	1270	1250	960*
Renal excretion of sodium (meq.).....			320	181		176	166*
Renal excretion of chloride (meq.).....			291	187		177	167*
Serum sodium (meq./liter)...		145	142	144		140	143†
Serum chloride (meq./liter)...		101	101	100		98	100†
Plasma protein (gm. %)....		6.60					6.83†
Hematocrit (%).....		44.0	47.9	46.3		48.0	44.9†
Plasma volume (cc.).....		3290					3060†
Blood volume (cc.).....		5870					5560†
Available fluid (NaSCN) (cc.).....		17000					16870†

\* For the 24 hour period ending at 8 a.m. on the date given.

† For a fasting state in the morning.

reflecting the variations in the temperature of the environment. The change in the skin temperature appeared to be complete within the twenty-four hour period following the change in the environment, and there was no evidence of more gradual variations which might be associated with the process of acclimatization.

The basal metabolic rate, determined daily for the subjects in experiment 2, demonstrated no significant alteration with the changing environment. The metabolism in calories per square meter per hour is recorded in tables 5 and 6.

**DISCUSSION.** The effects of the environmental temperature on the specific phases of the water balance mechanism, such as the fluid and electrolyte loss by sweating and the variation in the circulating blood volume, have been studied by

numerous investigators. The present work represents an investigation of the water balance under conditions more carefully controlled than have been previously attempted. Since ambient air temperature is only one of the factors influencing the channels through which heat is lost from the body, we considered it imperative to maintain constancy of the wall temperature, the relative humidity, and the air movement. It should be noted that the rather confining conditions of the experiment permitted the subjects only a small, but quite constant,

TABLE 5

Experiment 2, Subject C, male, 31 years, married, graduate student. Height 183 cm., Surface area 2.02 sq. m. Daily intake—sodium 185 meq., chloride 179 meq.

DECEMBER 1940	WARM PERIOD			COLD PERIOD		
	19th	20th	21st	22nd	23rd	24th
Morning nude weight 18th— 80182 (grams).....	80150	80279	80093	79830	79445	79360*
Water intake (cc.).....	3348	3296	3554	2595	2169	2519*
Renal excretion of water (cc.)..	1357	1162	1425	1900	1465	1475*
Extrarenal water loss (cc.)....	1980	1987	2204	851	1056	1080*
Renal excretion of sodium (meq.).....	143	123	163	197	176	189*
Renal excretion of chloride (meq.).....	162	128	164	195	189	203*
Serum sodium (meq./liter)....	142	148	146	147	147	146†
Serum chloride (meq./liter)...	104	103	106	102	103	103†
Plasma protein (gm. %).....		6.35				6.60†
Hematocrit (%).....	44.4	44.3	43.2	44.2	44.6	44.9†
Plasma volume (cc.).....		3660				3540†
Blood volume (cc.).....		6570				6440†
Available fluid (NaSCN) (cc.)..		23600				20400†
Cal/hr. (m <sup>2</sup> ) .....	34.7	35.9	37.0	34.7	38.2	35.9†
Mean skin temp. (°F.).....	93.9	92.9	94.3	87.3	86.2	86.3‡
Mean surface temp. (°F.).....	89.1	89.6	89.4	77.9	72.5	74.9‡

\* For the 24 hour period ending at 8 a.m. on the date given.

† For a fasting state in the morning.

‡ Late afternoon.

amount of physical activity, thereby maintaining for them a fairly constant daily metabolism.

Diuresis on exposure to cold has been noted by many observers, and this increased renal function has been considered indicative of the loss of body fluid (Burton et al., 1940). In all the experiments reported here a diuresis on exposure to cold was noted. This increased water loss may be a compensatory mechanism because, on exposure to cold, while the water loss in the sweat has been reduced the water intake in the diet may not have been sufficiently restricted. The measurement of the extracellular fluid space by the use of sodium

TABLE 6

Experiment 2, Subject D, male 22 years, single, medical student. Height 190 cm., Surface area 1.92 sq. m. Daily intake—sodium 185 meq., chloride 179 meq.

DECEMBER 1940	WARM PERIOD			COLD PERIOD		
	19th	20th	21st	22nd	23rd	24th
Morning nude weight 18th—66274 (grams).....	66126	66528	66114	66066	65960	65726*
Water intake (cc.).....	3030	3002	2972	2520	2125	2550*
Renal excretion of water (cc.)..	1280	938	1220	1600	1515	1717*
Extrarenal water loss (cc.)....	1805	1566	2023	810	635	942*
Renal excretion of sodium (meq.).....	164	115	182	198	168	189*
Renal excretion of chloride (meq.).....	175	126	176	208	179	189*
Serum sodium (meq./liter)....	145	150	147	145	144	147†
Serum chloride (meq./liter)...	105	102	103	101	101	103†
Plasma protein (gm. %).....		6.25				6.35†
Hematocrit (%).....	47.5	47.2	46.3	48.2	49.4	47.5†
Plasma volume (cc.).....		3050				2940†
Blood volume (cc.).....		5770				5600†
Available fluid (NaSCN) (cc.)..		19700				18500†
Cal/hr. (m <sup>2</sup> ).....	32.6	33.5	31.4	31.7	30.1	32.1†
Mean skin temp. (°F.).....	93.6	92.5	93.2	88.1	85.1	87.5†
Mean surface temp. (°F.).....	87.9	89.5	89.8	75.8	74.1	74.7†

\* For the 24 hour period ending at 8 a.m. on the date given.

† For a fasting state in the morning.

‡ Late afternoon.

TABLE 7

Experiment 3, Subject E, male, 21 years, single, medical student. Height 185 cm., Surface area 2.03 sq. m. Daily intake—sodium 177 meq., chloride 173 meq.

DECEMBER 1941	COLD PERIOD			WARM PERIOD		
	19th	20th	21st	22nd	23rd	24th
Morning nude weight 18th—79290 (grams).....	78479	78419	78355	77848	77768	77979*
Water intake (cc.).....	1922	1871	2653	3024	3365	3664*
Renal excretion of water (cc.)..	1658	880	1610	795	720	690*
Extrarenal water loss (cc.)....	974	962	1020	2714	2679	2726*
Renal excretion of sodium (meq.).....	202	120	148	128	106	105*
Renal excretion of chloride (meq.).....	195	120	153	111	107	97*
Serum sodium (meq./liter)....	142	143	138	141	141	144†
Serum chloride (meq./liter)...	96	100	100	99	99	103†
Hematocrit (%).....	54.4	51.3	50.6	49.5	48.7	45.8†
Plasma volume (cc.).....		3230				4170†
Blood volume (cc.).....		6630				7720†
Available fluid (NaSCN) (cc.)..		18000				18450†
Rectal temp. (°F.).....	99.2	99.2	100.0		99.6	99.8†
Mean skin temp. (°F.).....	81.5	85.8			94.0	93.9†
Mean surface temp. (°F.).....	75.7	76.9	77.2	92.5	91.6	93.9†

\* For the 24 hour period ending at 8 a.m. on the date given.

† For a fasting state in the morning.

‡ Late afternoon.

thiocyanate has provided no definite evidence of any appreciable decrease in this compartment on exposure to cold.

Under the conditions of these experiments the subjects' extrarenal water loss was quite constant from day to day in each constant temperature period. On changing the environmental temperature an immediate and pronounced change in the rate of extrarenal water loss was observed. This change in the extrarenal water loss appeared to be completed almost immediately, so that during the short period of time occupied by these experiments there was no evidence of a gradual acclimatization. These results differ somewhat from the findings of Burton et

TABLE 8

Experiment 3, Subject F, male, 22 years, single, medical student. Height 177 cm., Surface area 2.01 sq. m. Daily intake—sodium 177 meq., chloride 173 meq.

DECEMBER 1941	COLD PERIOD			WARM PERIOD		
	19th	20th	21st	22nd	23rd	24th
Morning nude weight 18th—83051 (grams).....	83003	82600	82228	82163	82215	82246*
Water intake (cc.).....	2138	1848	2670	3445	3480	3498*
Renal excretion of water (cc.)	1240	1250	1827	717	530	535*
Extrarenal water loss (cc.)....	929	974	1077	2719	2884	2781*
Renal excretion of sodium (meq.) .....	161	170	166	89	49	55*
Renal excretion of chloride (meq.).....	167	169	157	90	60	66*
Serum sodium (meq./liter)....	140	145	141	140	139	142†
Serum chloride (meq./liter)...	101	105	103	101	101	103†
Hematocrit (%).....	46.9	47.6	47.7	46.4	45.2	43.0†
Plasma volume (cc.).....		3315				3800†
Blood volume (cc.).....		6330				6670†
Available fluid (NaSCN) (cc.)		18725				18725†
Rectal temp. (°F.).....	99.0	98.6	99.3		99.8	100.0†
Mean skin temp. (°F.).....		85.2			93.5	93.1†
Mean surface temp. (°F.).....	77.1	75.6	77.1	92.2	91.7	92.8‡

\* For the 24 hour period ending at 8 a.m. on the date given.

† For a fasting state in the morning.

‡ Late afternoon.

al. (1940), who noted a supernormal evaporative loss on the first day of exposure to cold with subnormal values appearing a few days later.

The large increases in the plasma volume (15 and 29 per cent) observed in subjects E and F on exposure to heat are of the same order of magnitude as those found by other investigators (Bazett et al., 1940; Maxfield et al., 1941). The decreases in plasma volume of 3, 4, 7, and 8 per cent as measured after three days' exposure to cold are in general smaller than the changes, 2, 12, 13, 22, and 32 per cent, observed by Bazett et al. under similar conditions. In the present experiments the plasma volume appeared initially to have decreased on exposure to cold to the extent observed by Bazett, but, when measured on the third day of cold, it had returned toward the control value.



## SUMMARY

1. The effect of changes in the external temperature on the fluid and electrolyte balance has been studied on six normal male subjects under carefully controlled dietary and environmental conditions.

2. The extrarenal water loss was quite constant during any period of uniform temperature. Upon change of the environmental temperature, the extrarenal water loss and the skin temperature quickly attained new levels. There was no evidence of slow acclimatization.

3. The renal excretion of sodium and chloride appeared to reflect the difference between the intake of these substances and the quantities lost in the sweat.

4. A pronounced and prolonged increase in plasma volume was found on exposure to heat. On exposure to cold the plasma volume was reduced but tended to return toward normal within a few days.

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# RESPONSE OF RATS TO BORON SUPPLEMENTS WHEN FED RATIONS LOW IN POTASSIUM<sup>1</sup>

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The demonstration that boron is an essential element in the nutrition of plants and the accumulation of data showing its occurrence in animal tissues have served as stimuli for investigations to ascertain whether this element is essential in the metabolism of animals. Boron is held so tenaciously by those materials ordinarily used in compounding synthetic rations that investigators (1-3) have had to rely upon rations containing appreciable amounts of the element. Lack of agreement among the reports regarding response to boron supplementation is therefore not surprising.

That the requirements of the animal body for a given element may be markedly influenced by the concentration of one or more additional elements in the ration is well illustrated by the necessity for a proper ratio between calcium and phosphorus in the diet. Such interrelationships in the plant kingdom between boron and other elements are well known. Boron deficiency in alfalfa, for example, is most likely to appear on soils which have been limed to an excess. Reeve and Shive (4, 5) in their study of the influence of potassium upon boron metabolism in plants observed that boron was absorbed poorly from media in which the concentration of potassium was quite low. If boron is an essential element in the metabolism of animals, one may reasonably assume that in animals, as in plants, its functions are interrelated with those of other elements. Furthermore, development of symptoms of boron deficiency should be facilitated by feeding rations of such composition that the body would be unable to maintain these important interrelationships. Using a basal ration of low potassium content we have obtained data which indicate that boron, at least under these conditions, plays a rôle in animal nutrition although there was no evidence of an interrelationship between these two elements in the animal body.

**PROCEDURE.** Animals with limited stores of boron were prepared by placing stock colony rats with their litters, when the latter were two weeks of age, in cages provided with wire false bottoms and feeding them a diet of whole milk until the young weighed 40 to 50 grams. The litters were then divided so as to provide a litter mate control of the same sex and approximately the same weight for each animal receiving a boron supplement. The rats were housed in individual wire cages.

<sup>1</sup> The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the Director. A preliminary report was made before the Agricultural and Food Division of the American Chemical Society at its 107th meeting.

The basal ration, which contained 0.45 p.p.m. of boron and 51 p.p.m. of potassium, had the following percentage composition: crude casein, 18; sucrose, 75.7; salts,<sup>2</sup> 5; fortified corn oil,<sup>3</sup> 1; and a mixture<sup>4</sup> of B vitamins, 0.3. Boron additions were made by mixing weighed amounts of either boric acid or borax with portions of the basal ration. Dipotassium phosphate was incorporated in the same way when adding a potassium supplement. The rations were fed *ad libitum*.

The greater number of the animals used in the study were fed their respective rations until death resulted from potassium deficiency. Others, after differing lengths of time on the rations, were killed and analyzed for liver glycogen and body fat. The method of Good, Cramer, and Somogyi (6) was used for liver glycogen, and body fat was determined by ether extraction of the eviscerated and dried carcasses. In order to ascertain the extent to which boron intake influenced storage of the element, about half of the ether-extracted samples were analyzed for this element by the Berger and Truog procedure (7).

**RESULTS.** It should be mentioned at the outset that very little growth was possible on rations containing such low concentrations of potassium. Animals weighing initially 45 grams seldom exceeded 55 grams in weight. On the other hand there was no appreciable loss of weight. In fact, because of accumulation of fluid in the intestinal tract, the animals usually weighed a few grams more at death than when placed on the experimental ration.

The survival periods of a total of 54 pairs of rats under seven sets of conditions as regards supplementation of the basal ration are recorded in figure 1 and a summary thereof is presented in table 1.

Boric acid was used as a boron supplement for groups I-IV, borax for groups V-VII. For a given pair of animals the difference in survival periods was considered to be significant only when one exceeded the other by as much as 10 per cent. It will be noted in table 1 that boron exerted a beneficial effect upon six of the seven groups of rats. In two of the groups—II and VI—no control animal lived significantly longer than its high-boron mate. Although 1,000 p.p.m. of boron in the form of borax was without apparent effect in group V, in the preliminary investigations previously reported five of the six animals receiving this supplement responded favorably. Considering the data for all groups, there were 33 instances in which survival periods of the boron-fed animals exceeded those of the respective controls by as much as 10 per cent, whereas in 8 instances they were appreciably shorter. In thirteen pairs the mates survived approximately the same number of days. In order to terminate the experiment the following high-boron animals were discarded after the time intervals indicated on the chart: 8 of group IV, 9 of group VI, and 6 and 7 of group VII.

<sup>2</sup> The salt mixture was that of Phillips and Hart, J. Biol. Chem. 109: 657, 1935, except that the concentrations of copper and manganese were doubled and dipotassium phosphate was replaced with an equal weight of monosodium phosphate.

<sup>3</sup> Carotene, 0.133 gram; viosterol, 1 gram; alpha-tocopherol, 0.4 gram; 2-methyl-1,4-naphthoquinone, 10 mgm.; corn oil, 100 grams.

<sup>4</sup> Thiamine hydrochloride, 20 mgm.; pyridoxine hydrochloride, 20 mgm.; riboflavin, 50 mgm.; calcium pantothenate, 0.1 gram; nicotinic acid, 5 grams; p-aminobenzoic acid, 5 grams; inositol, 10 grams; choline chloride, 10 grams.

That there was a noticeable effect of boron upon survival by the twenty-fifth day and a pronounced effect after the thirtieth day is evident upon comparison of the values in the lower half of table 1. Nineteen controls as compared with 12 high-boron rats were dead on or before the twenty-fifth day. At the other extreme only one control survived as long as 60 days as compared with 9 in the

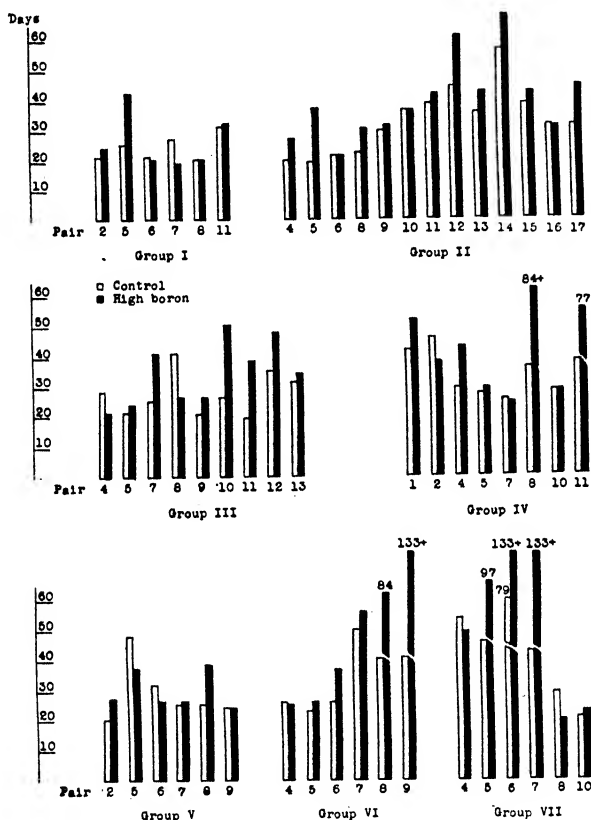


Fig. 1. Effect of boron intake upon survival on low-potassium rations. The basal diet, containing 0.45 p.p.m. boron and 51 p.p.m. potassium, was supplemented as follows:

- Group I Control, none; high-boron, 100 p.p.m. B as boric acid.
- Group II Control, none; high-boron, 500 p.p.m. B as boric acid.
- Group III Control, none; high-boron, 250 p.p.m. B as boric acid.
- Group IV Control, none; high-boron, 1000 p.p.m. B as boric acid.
- Group V Control, none; high-boron, 1000 p.p.m. B as borax.
- Group VI Control, 100 p.p.m. K; high-boron, 100 p.p.m. K and 1000 p.p.m. B as borax.
- Group VII Control, 250 p.p.m. K; high-boron, 250 p.p.m. K and 1000 p.p.m. B as borax.

boron groups. Furthermore, 6 high-boron animals, representing 11 per cent of the total number, were living after all of the 54 control animals had died.

Since supplementation of low-potassium rations with boron, although failing to promote growth, enabled rats to survive for longer periods, it appeared probable that the element exerted an influence upon either storage of energy-yielding reserves or the reactions by which energy was released from these reserves. For

this reason the investigation was extended to determination of blood sugar, liver glycogen and body fat after the animals had been on the variously supplemented rations for differing lengths of time. Since analysis of blood for sugar content was discontinued early in the investigation when preliminary analyses failed to show any correlation between this body constituent and boron intake, no data for blood sugar are recorded. In column 5 of table 2 it will be noted that the concentration of liver glycogen in the boron-fed rats exceeded that of the controls at 3 and 14 days and in 5 of the 7 groups analyzed at 21 days. The highest value, 6.70 per cent, was obtained in the boron animals of group VII. Moreover, in column 5 all of the values above 5 per cent were found in livers of boron animals. The concentration of liver glycogen at 21 days averaged 3.11 per cent for controls

TABLE 1  
*Summary of data presented in figure 1*

	CONTROL	HIGH-B
Number living significantly longer than pair mates		
Group I.....	1	2
Group II.....	0	9
Group III.....	2	7
Group IV.....	1	4
Group V.....	2	2
Group VI.....	0	5
Group VII.....	2	4
Total.....	8	33
Number of animals employed.....	54	54
Number dead on or before 25th day.....	19	12
Number surviving 30 or more days.....	25	33
Number surviving 40 or more days.....	13	21
Number surviving 50 or more days.....	4	12
Number surviving 60 or more days.....	1	9
Number surviving 70 or more days.....	1	7
Number surviving 80 or more days.....	0	6

as compared with 4.57 for the animals receiving the two boron supplements. Hence the livers of boron-fed rats contained on the average 47 per cent more glycogen than their control mates.

Since there had been no attempt previously to control the intake of ration before the rats were killed for analysis, it was deemed advisable to analyze livers of rats which had been fasted and then given known amounts of sugar. In order to grow the animals to a size such that they could take the stomach tube which was available for use, a suboptimal amount of potassium, 750 p.p.m., was added to the basal ration of eight low-boron rats for approximately six weeks. They were then fasted for 24 hours, after which they were given by stomach tube 2 cc. of solution containing 0.5 gram of glucose. The solution given four of the rats contained in addition to the sugar 500 p.p.m. of boron as boric acid. Two hours after administration of the sugar solution the animals were killed and liver

glycogen was determined. Eighteen per cent more glycogen was found in the livers of rats receiving the boron-glucose solution than in those receiving only glucose, the amounts averaging 48.5 and 41.2 mgm., respectively. The concentrations in the livers of the two groups as named were 1.65 and 1.36 per cent.

TABLE 2

*Influence of boron intake upon concentration of liver glycogen, body fat and boron in rats\* fed a ration deficient in potassium*

GROUP	SUPPLEMENTS		DAYS ON RATION	LIVER GLYCOGEN (WET BASIS)	BODY FAT (DRY BASIS)	BODY BORON (DRY BASIS)
	Potassium	Boron†				
	<i>p.p.m.</i>	<i>p.p.m.</i>		<i>per cent</i>	<i>per cent</i>	<i>p.p.m.</i>
A	0	0	3	4.28	31.7	
	0	1,000	3	5.41	28.4	
B	0	0	7	4.85	22.7	
	0	1,000	7	4.70	22.1	
C	0	0	10	4.97	11.8	
	0	1,000	10	4.67	15.0	
D	0	0	14	2.97	11.1	
	0	1,000	14	4.39	15.5	
I	0	0	21	4.33	7.6	1.17
	0	100	21	5.84	7.4	6.94
II	0	0	21	3.68	8.5	0.91
	0	500	21	5.22	9.9	21.04
III	0	0	21	2.61	7.0	1.16
	0	250	21	3.83	8.2	8.95
IV	0	0	21	1.13	7.7	
	0	1,000	21	4.34	10.5	
V	0	0	21	3.25	9.3	1.59
	0	1,000	21	3.56	10.6	26.86
VI	100	0	21	2.71	10.5	
	100	1,000	21	2.48	9.3	
VII	250	0	21	4.08	9.2	1.27
	250	1,000	21	6.70	9.0	38.20

\* Each analysis represents the average of three animals.

† Boric acid was used in groups A to IV and borax in groups V, VI and VII.

That the experimental animals quickly lost the larger part of their storage fat when placed on the low-potassium ration is shown by inspection of column VI of table 2. Whereas litter mates of the experimental animals when weaned contained 36 per cent body fat, the low-boron animals in this column contained 31.7,

22.7, 11.8 and 11.1 per cent fat after 3, 7, 10 and 14 days, respectively, on the basal ration. Of the seven groups analyzed after 21 days on the low-potassium rations there were four—groups II, III, IV and V—in which boron supplementation favored maintenance of stores of body fat whereas in only one instance—group VI—did the boron-fed rats contain appreciably less fat than their control mates. For the seven groups analyzed at 21 days the low-boron animals averaged 8.5 per cent fat as compared with 9.3 per cent for the high-boron mates.

Boron analyses for some of the animals are recorded in column VII merely to indicate the concentrations which resulted from feeding boron at the widely differing levels of intake. In the low-boron animals the concentration of this element ranged from 0.91 to 1.59 p.p.m. with an average of 1.22 p.p.m. In the groups fed boric acid supplements at boron concentrations of 100, 250 and 500 p.p.m. of ration for 21 days the respective concentrations of the element in the carcasses were 6.94, 8.95 and 21.04 p.p.m. The highest concentration, 38.20 p.p.m., was found in those rats which in addition to being given 1000 p.p.m. of boron were also given a small supplement of potassium.

Since responses to boric acid and borax supplements were so much alike as regards concentration of liver glycogen and body fat, the effects exerted by these two compounds apparently were due to the boron contained therein. Probably the increase in survival periods observed when the low-potassium ration was supplemented with boron compounds was due to the effect of boron upon maintenance of these energy-reserves in the body. It appears likely that boron is able to substitute in a limited way for potassium in certain rôles which the latter element performs in the body, perhaps in connection with certain enzyme systems. That absorption of boron was not impaired appreciably by lack of adequate potassium in the diet is indicated by the fact that the control animals in groups I, II, III and V, which received no potassium supplements, contained an average of 1.22 p.p.m. of boron as compared with 1.27 in the control animals of group VII for which the potassium supplement, 250 p.p.m., increased the intake of this element five-fold.

#### SUMMARY

Rats consuming low-potassium rations supplemented with either boric acid or borax survived longer than pair mates which received no boron.

After 21 days on the low-potassium rations rats fed boron supplements contained in their livers 47 per cent more glycogen than those receiving no additional boron.

Maintenance of stores of body fat was favored by liberal supplementation of the low-potassium rations with boron compounds.

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# THE EFFECT OF ANOXIA ON FAT ABSORPTION IN RATS

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There is considerable evidence that anoxia is capable of influencing absorption from the small intestine (1). Its effect on the rate of absorption may differ not only for different substances, but with varying degrees of anoxia as well. It was shown, for example, by Northup and Van Liere (2) that in dogs subjected to moderately severe degrees of anoxia (partial pressures of oxygen down to 63 mm. Hg) the absorption of glycine and of glucose was practically unchanged (from normal). At more severe degrees of anoxia (partial pressure of oxygen of 53 mm. Hg), however, the rate of absorption of glycine was significantly depressed whereas glucose tended to be absorbed more rapidly.

Although direct studies on the effect of anoxia on the absorption of fat have not been reported, as far as the author is aware, some work has been carried out on the effect of reduced atmospheric pressure on the level of the blood lipids. Starup (3) reported that prolonged exposure to reduced atmospheric pressure produced a marked lipemia in rabbits. MacLachlan (4) found that short periods of reduced atmospheric pressure had no effect on the blood plasma lipids of cats or dogs either in the fasting state or during active absorption of fat from the intestine. Rabbits, on the other hand, showed a definite lowering of the lipid content of the plasma, due mainly to a decrease in the neutral fat fraction. Sundstroem and Bloor (5) state that a decrease in the lipid phosphorus of the plasma is a characteristic feature of short exposure to low atmospheric pressure.

The rate of absorption of fat from the alimentary tract as measured indirectly by following the changes in the level of the blood lipids is, of course, influenced by factors other than the actual absorption of fat from the intestinal tract as, for example, the rate of metabolism, deposition of fat in the tissues, and mobilization of fat from the tissues. Obviously, the direct measurement of the rate of fat absorption by determining the amount of fat remaining in the alimentary tract at intervals after feeding fat is preferable. In the present investigation the direct method was employed to determine the effect of anoxia on the absorption of fat in rats.

**EXPERIMENTAL.** The technique developed by Irwin and co-workers (6) for determining the rate of absorption of fat was followed. In applying this technique certain modifications were made which are described in detail. Adult albino rats of both sexes, weighing between 150 and 350 grams, were fasted 48 hours and given 1.5 cc. of corn oil ( $1.385 \pm 0.01$  gram) by stomach tube under light ether anesthesia. After fully recovering consciousness (1-2 min.) the experimental rats were placed in a steel respiratory chamber, described by Van Liere (7), and subjected to the following partial pressures of oxygen: 117, 80,



63, and 53 mm. Hg (corresponding approximately to altitudes of 8,000, 18,000, 24,000, and 28,000 ft., respectively). Simultaneously fed controls were kept at atmospheric pressure. Four hours after feeding, the amount of unabsorbed fat in the stomach, small intestine and cecum was quantitatively determined and the value used to calculate the per cent absorbed, correction being made for the fat present in the fasted gut.

Manual stripping of the intestine to remove the last vestige of fat, as described in the technique of Irwin et al. (6), proved difficult owing to the ease with which the gut would break, especially the upper portion. Moreover, care had to be exercised to prevent fatty tissue which adhered to the outer surface of the intestine from rubbing off. These difficulties were satisfactorily solved by the following procedure. The strip of intestine was placed at full length on an elevated plate-glass platform so that one end of the gut hung over the edge of the plate-glass into the mouth of a 125 cc. Erlenmeyer flask fitted with a small funnel. Keeping the other end of the strip of intestine closed, the contents could be quickly and completely evacuated into the flask by compressing the full length of intestine by the use of a sponge rubber roller (about 2.5 in. in diameter).

Extraction of the fat from the gastrointestinal contents was carried out in the 125 cc. Erlenmeyer flask, equipped with a ground glass stopper. The combined contents of the stomach, small intestine and cecum, together with the collected washings with water and petroleum ether, were heated on the steam bath for several minutes and the warm petroleum ether layer carefully decanted into a 50 cc. centrifuge tube. The extraction was repeated three times with 10 to 15 cc. portions of petroleum ether, the contents being shaken vigorously each time before heating on the steam bath. (Caution must be exercised in removing the stopper from the extraction flask after shaking the warm contents with petroleum ether due to the internal pressure which develops. A small amount of petroleum ether applied to the outer surface of the flask quickly reduces the pressure.) The combined petroleum ether extracts were centrifuged to remove any sediment and quantitatively transferred to a tared 125 cc. Erlenmeyer flask. The solvent was removed by evaporation on the steam bath, the last traces being blown out with a gentle current of nitrogen. The flask was then allowed to stand overnight in a vacuum desiccator with the pressure lowered to 80 mm. Hg, in an oven at 70°C. After cooling in the desiccator the flask was weighed.

To test the accuracy of this technique 10 rats were fasted 48 hours and fed 1.5 cc. of corn oil, killed immediately, and the fat recovered from the alimentary tract by the method described. The mean percentage recovery was 96.3 (range 94.5 to 98).

In calculating the per cent of fat absorbed it was necessary to correct for the fat present in the intestinal tract after the 48 hour fast. Eight rats were, therefore, fasted, killed and the fat recovered by the method described above. The mean weight of fat obtained was 0.0257 gram. This approximates closely the value (0.0222 gram) obtained by Irwin and co-workers (6) for rats.

**RESULTS AND DISCUSSION.** In table I are given the data on the amount of fat absorbed by rats four hours after the administration of 1.5 cc. of corn oil ( $1.385 \pm$

0.01 gram). In all 74 animals were used. At first glance it might appear that the range (39.6 to 57.1) in the per cent absorption of fat for the control animals is large. However, as pointed out by Irwin and associates (6), such variations are usually encountered in experiments on absorption of fat, although the extreme values seldom fall outside the range of  $M \pm 10$  to 12. These variations are attributable in part to innate differences in the ability of the animals to absorb fat, as well as to possible differences in the physiological condition of the rats referable to the time at which the experiments were conducted. Accordingly it was considered advisable to confine comparisons to experiments carried out at the same time on experimental and control animals.

No correlation was apparent between the sex or body weight of the rats and the amount of fat absorbed. This is in agreement with the observations of Irwin et al. (6).

The amount of fat absorbed by rats subjected to partial pressures of oxygen of 117 and 80 mm. Hg (38.2 and 41.4 per cent, respectively), was not significantly

TABLE 1

*Effect of anoxia on the absorption of fat from the small intestine in rats*

OXYGEN TENSION .....		117 MM.	80 MM.		63 MM.		53 MM.	
Approximate altitude .....		8,000 ft.	18,000 ft.		24,000 ft.		28,000 ft.	
	No. of rats	Fat absorbed	No. of rats	Fat absorbed	No. of rats	Fat absorbed	No. of rats	Fat absorbed
		%		%		%		%
Controls.....	9	39.6	7	47.1	8	41.5	10	57.1
Anoxic.....	9	38.2	10	41.4	11	28.6	10	31.8
Std. Dev.....		8.5		8.3		8.8		11.8
P (Fisher's).....		0.8		0.2		<0.01		<0.001

different from that for the control animals (39.6 and 47.1 per cent, respectively). On the other hand, at partial pressures of oxygen of 63 and 53 mm. Hg the amount absorbed was significantly less (28.6 and 31.8 per cent, respectively) than for the corresponding controls (41.5 and 57.1 per cent, respectively). The difference, statistically, was highly significant.

These results appear to corroborate the observation of MacLachlan (4) that in rabbits, subjected to a reduced atmospheric pressure of 354 mm. Hg (partial pressure of oxygen of 74 mm. Hg) for three hours, there is a definite lowering of the total lipid content of the plasma. Although the decrease was confined mainly to the neutral fat fraction, the phospholipid showed some tendency to decrease as well. Sundstroem and Bloor (5) reported that a decrease in the lipid phosphorus of the plasma is a characteristic feature of short exposure to low pressure.

Apparently the threshold for the absorption of fat in the rat lies between partial pressures of oxygen of 80 and 63 mm. Hg (corresponding approximately to altitudes of 18,000 and 24,000 ft., respectively). It is of interest to note that Van Liere, David and Lough (8) found that the absorption of water in dogs was not affected by anoxia until a partial pressure of oxygen of 80 mm. Hg (18,000

ft.) was reached. At this partial pressure of oxygen considerably more water was absorbed by the anoxic dogs. Although no adequate explanation was offered it was pointed out that this occurred at what Barcroft has termed the critical level of anoxia.

There is considerable evidence that anoxia results in a diminished motility of the stomach and small intestine, as well as a prolongation of the emptying time of the stomach, in both man and dogs (1). These factors might reasonably be expected to affect significantly the rate of fat absorption.

Steenbock and associates (9) observed a slowing up of fat absorption in rats as a result of inadequate intake of vitamins, or reserves of vitamins. Other abnormal body conditions such as anemia also caused a decided decrease. These findings were interpreted as an expression of a general slowing down of body activities. The decreased absorption of fat in rats subjected to severe degrees of anoxia might likewise be referable to a diminished rate of cellular metabolism resulting from a decreased supply of oxygen to the tissues.

The results of this investigation on the effect of anoxia on fat absorption show that ranges of anoxia compatible with life would not interfere, as far as the absorption of fat is concerned, with the proper nourishment of the body. However, as pointed out by Van Liere (1), these studies in absorption are valuable in so far as they may throw additional light on the mechanism of the absorptive processes.

#### SUMMARY

Adult albino rats, previously fasted 48 hours, were fed 1.5 cc. of corn oil ( $1.385 \pm 0.01$  gram) and subjected to the following partial pressures of oxygen: 117, 80, 63 and 53 mm. Hg (corresponding approximately to altitudes of 8,000, 18,000, 24,000 and 28,000 ft., respectively). Simultaneously fed controls were kept at atmospheric pressure. Four hours after feeding the amount of fat absorbed was determined by measuring the amount of fat remaining in the alimentary tract.

The amount of fat absorbed by rats subjected to partial pressures of oxygen of 117 and 80 mm. Hg was not significantly different from that for the control animals. On the other hand, at partial pressures of oxygen of 63 and 53 mm. Hg the amount absorbed was significantly less than for the corresponding controls. The difference, statistically, was highly significant. Apparently the threshold for the absorption of fat in the rat lies between partial pressures of oxygen of 80 and 63 mm. Hg.

The results of this study indicate that ranges of anoxia compatible with life would not interfere, as far as the absorption of fat is concerned, with the proper nourishment of the body.

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# THE CARDIAC RESPONSE TO STIMULATION OF THE STELLATE GANGLIA AND CARDIAC NERVES<sup>1</sup>

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In a previous paper (1), it was reported that in the anesthetized dog, faradic stimulation of the stellate ganglia or their cardiac branches causes coronary inflow to increase. Early in these experiments, it was observed that stimulation of these nerve structures affected the heart in such a manner that its vigor of contraction appeared to increase greatly. Although increases in heart rate and blood pressure were also frequently observed, they were not always present and therefore could not be considered indispensable to the mechanism by which the coronary flow was increased. If, judging from appearances alone, the work output of the heart were being increased by nerve stimulation, a concomitant increase in coronary flow would be a reasonable expectation. Accordingly, further experiments have been designed to determine, if possible, the mechanism by which stimulation of these sympathetic structures leads to an increase in coronary flow. The studies have been largely restricted to the measurement of those factors concerned with the work and metabolism of the heart.

**METHODS.** The preparation was essentially the same as that used in the previous nerve experiments (1). Dogs weighing from 9.5 to 12.5 kilos. were anesthetized<sup>3</sup> and the upper part of the chest plate removed. By careful dissection, the stellate ganglion and/or its cardiac branches were exposed on one or both sides. One main branch (descendens or circumflex) of the left coronary artery was isolated 8 to 10 mm. from the aorta for subsequent cannulation. One common carotid artery was isolated and anticoagulants<sup>4</sup> were then given. In some preparations, an adjustable screw clamp was applied to the thoracic aorta near the diaphragm so that central aortic pressure could be regulated if desired. Coronary inflow was measured by a rotameter (2) inserted between the carotid cannula and the coronary artery cannula. In some experiments the flow was optically recorded by a special rotameter and optical recording attachment which are described elsewhere (3, 4). The nerve structures were stimulated with a Harvard inductorium.

Measurements of cardiac input were made by means of a large bore rotameter attached to a special cannula<sup>5</sup> inserted into both venae cavae. Blood leaving

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<sup>3</sup> Sodium pentobarbital, 0.020 gm./kilo.

<sup>4</sup> Heparin, 100 units/kilo. and pontamine fast pink, 150 mgm./kilo.

<sup>5</sup> See reference (5) for description and use of cannula. The azygos and any other veins not included in the cannulation were tied off. In these experiments, venous blood returning from the coronary circuit was not included in the measurement of cardiac input.

the rotameter was returned to the right atrium through a cannula tied in the tip of the right auricular appendage.

A-VO<sub>2</sub> differences were determined from blood samples drawn from a side tube on the coronary artery cannula and from a needle inserted into the great cardiac vein.

In figure 1 are illustrated the usual temporal relations between the time of cardiac nerve stimulation and the response of coronary inflow and blood pressure (uncompensated).

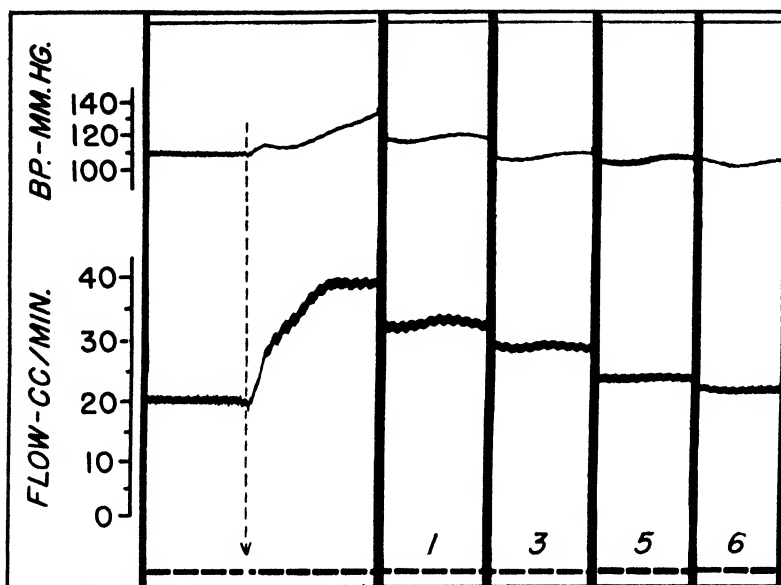


Fig. 1. Segments of original record showing one type of response to cardiac nerve stimulation. Upper curve, mean carotid arterial pressure. Lower curve, flow through circumflex branch of left coronary artery. Time, 5 seconds. Ordinate, pressure in mm.Hg and flow in cc/min. Interrupted vertical line indicates beginning stimulation. Numbers at bottom of record segments indicate minutes elapsed following cessation of stimulation.

*Work changes with cardiac nerve stimulation.* In part A of table 1 are presented four sets of observations which are typical of repeated determinations on each of three animal (9-10 kilo.) preparations. In experiments 1, 2 and 3, any elevation in blood pressure during cardiac nerve<sup>6</sup> stimulation was prevented by loosening the aortic clamp which had previously been adjusted to constrict the aorta slightly. In experiment 4, the blood pressure was not artificially adjusted. Since, in each instance, cardiac input is significantly increased while the blood pressure remains the same or increases, the work of the left ventricle must also have increased as the result of cardiac nerve stimulation.

<sup>6</sup> The expression "cardiac nerves" used throughout the paper refers to the nerve branches from the stellate ganglion which are directed toward the heart.

It has been reported in a previous publication (1) that right coronary inflow also increases when the cardiac nerves are stimulated. In all of nine experiments in which right ventricular pressure was optically recorded, the maximal systolic pressure rose considerably during nerve stimulation with increases ranging from 44 to 200 per cent, average 89 per cent. The observations that right ventricular pressure increases and cardiac input increases must indicate that the work of the right ventricle is also increased by cardiac nerve stimulation.

TABLE 1

*The effect of cardiac nerve stimulation on the work and metabolism of the left heart*  
Work changes

EXPT.	MEAN BLOOD PRESSURE-MM. Hg		CORONARY FLOW CC./MIN.		CARDIAC INPUT CC./MIN.		CONDITION
	Before	During	Before	During	Before	During	
1	70	69			760	1087	Left cardiac nerves stimulated 2 minutes
2	81	80	28	34	575	670	Right cardiac nerves stimulated 1 minute—Flow in left circumflex
3	106	108	32	47	350	500	Left cardiac nerves stimulated 1 minute—Flow in left circumflex
4	72	83	29	37	400	475	Left cardiac nerves stimulated $\frac{1}{2}$ minute—Flow in left circumflex

Metabolism changes

EXPT.	HEART RATE	MEAN BLOOD PRESSURE	O <sub>2</sub> CONTENT—VOL. %		A-VO <sub>2</sub> DIFFERENCE	CORONARY INFLOW		O <sub>2</sub> UTILIZATION		CONDITION
			Art.	Venous		cc./min.	% increase	cc./min.	% increase	
1	132	110	17.90	4.55	13.35	16		2.14		Control-flow in left circumflex
	138	108	17.80	3.46	14.34	32	100	4.59	114	Stim. left cardiac nerves 2 min.
2	120	110	16.0	2.98	13.02	17		2.21		Control-flow in left circumflex
	120	110	15.7	2.35	13.35	45	165	6.00	171	Stim. left cardiac nerves for 2.5 min.

*Metabolism changes with cardiac nerve stimulation.* As would be anticipated, and in keeping with the observed increase in cardiac work, the O<sub>2</sub> utilization of the heart is also increased with stimulation of the cardiac nerves. In table 1, part B, are presented two representative sets of data which reveal that the A-VO<sub>2</sub> difference increases slightly and coronary inflow increases greatly; the product of the two changes indicates a large increase in O<sub>2</sub> utilization.

**DISCUSSION.** Faradic stimulation of either right or left stellate ganglion or its cardiac branches, before or after severing them from the sympathetic chain, causes a considerable and sustained increase in both right and left coronary in-

flow with or without an appreciable accompanying increase in heart rate or blood pressure. Measurements of cardiac input and  $O_2$  utilization reveal that stimulation of these nerve structures causes an increase in the work and metabolism of the heart. If the nerves are unduly traumatized, incident to their isolation, or if the experiment is prolonged for several hours, nerve stimulation may be accompanied by only a small increase or no change in coronary inflow. In none of the experiments has the coronary inflow been found to increase without evidence of increased cardiac work and/or metabolism. In those experiments in which only coronary inflow was measured, an increase in flow was never observed without gross visual evidence that the vigor of cardiac contractions had also increased. In no experiment was a decrease in coronary flow observed during cardiac nerve stimulation. The foregoing observations support the conclusion that stimulation of the cardiac nerves causes the heart to beat more vigorously, thereby increasing cardiac output and/or blood pressure (work). The increase in work is accompanied by an increase in coronary A- $VO_2$  difference and an increase in coronary flow ( $O_2$  utilization and metabolism).

The mechanism by which the vigor of cardiac contraction is increased has not been identified. The possibility that adrenal secretion is responsible for the cardiac stimulating effect can be largely discounted for several reasons: 1, stimulation of the cardiac nerves when severed from the stellate ganglia gives the same cardiac response as stimulation of the stellate ganglia when the nerves and sympathetic trunk are intact; 2, the cardiac response occurs within several seconds (cf. fig. 1) which interval would not appear to be sufficiently long for elaboration and transportation of epinephrine from the adrenal glands; 3, in each of two animals, no diminution or alteration of the cardiac response was noted in observations made before and after the adrenals were clamped off.

Because of the promptness (1-3 sec.) with which the heart responds, it would appear more likely that the physiological process is a local one, perhaps involving the release of a "myocardial stimulating substance" at the endings of the stimulated nerves. Such a possibility is indirectly supported by several studies. Woolard (6) reports that certain nerves in the myocardium pass among the muscle fibres and many ultimately penetrate individual muscle cells. Cannon and Rosenbluth and others (7, 8, 9) offer evidence that an adrenalin-like substance ("sympathin") is released in the heart when the cardiac nerves are stimulated. Regardless of the immediate effect of nerve stimulation on the myocardium, it is not improbable that the consequent increase in cardiac vigor and work and the accompanying increase in metabolism are largely responsible for the dilatation of the coronary vessels and the observed increase in flow. Two possible mechanisms are suggested, either or both of which may operate; vasodilatation of the coronary bed resulting from 1, the increased local production and release of metabolites, and 2, the creation of a local relative anoxia caused by a disproportion between the increased rate of  $O_2$  utilization and existing coronary blood flow.

Thus far, the ultimate effect of cardiac nerve stimulation has been interpreted only in relation to what are regarded as the primary antecedents to the change in



coronary flow, namely, increases in cardiac work and metabolism. Previous investigators have interpreted the changes in coronary flow in terms of direct vasomotor influences on the coronary vessels. Katz and Jochim (10) have presented experiments with the fibrillating heart which were thought to demonstrate the effects of nerve action on the calibre of the coronary blood vessels without interference from concomitant alterations in blood pressure, vigor of contraction, etc. The observations presented here do not exclude the possibility that the cardiac nerves may exert a direct vasomotor influence on the coronary vessels. However, the studies with the fibrillating heart (10) also fail to demonstrate conclusively that such direct vasomotor influence does exist. The abolition of rhythmic contraction of the heart does not preclude the possibility that cardiac nerve stimulation may yet increase the vigor of fibrillary contractions, increase the metabolism and establish the same train of events as that observed when the myocardial fibres are undergoing concerted rhythmic contraction. The present studies on the beating heart have demonstrated increases in cardiac work and metabolism which, in themselves, are sufficient to explain the coronary dilatation and increased flow which accompany nerve stimulation. The failure to observe an increased coronary flow without evidence of increased cardiac vigor, work or metabolism lends indirect support to the thesis that the cardiac nerves are not primarily or even necessarily concerned with efferent vasomotor regulation by direct action.

Previous investigators have stressed the importance of the cardiac nerves in relation to reflex mechanisms "by which coronary flow can be adapted to the requirements of the heart." In the light of the present findings, the cardiac nerves would appear to operate in conjunction with a mechanism by which *the work output of the heart can be adapted to the requirements of the whole organism*. In the absence of conclusive evidence to the contrary, the concomitant increase in coronary flow is regarded largely as a secondary phenomenon resulting from coronary dilatation occasioned by chemico-metabolic influences incident to the increased work and metabolism.

#### SUMMARY

In confirmation of previous work, faradic stimulation of either stellate ganglion or its cardiac branches in the anesthetized open-chest dog causes a considerable and sustained increase in coronary flow. Measurements of cardiac input and  $O_2$  utilization have demonstrated that cardiac work and metabolism are also increased.

The cardiac nerves arising from the stellate ganglia are believed to be primarily involved in a mechanism by which the work output of the heart may be increased by nervous control. The promptness of the cardiac response and the somewhat prolonged after-effect suggest the elaboration of a cardiac stimulating substance at the endings of the stimulated nerves.

The failure to obtain an increased coronary flow response without evidence of increased cardiac vigor, work or metabolism, the lack of conclusive evidence that the cardiac nerves have a direct vasomotor influence and the observation that

cardiac metabolism is considerably increased, all lend support to the thesis that the increase in coronary flow may be largely, if not entirely, a secondary phenomenon. The increased vigor of cardiac contraction and the associated increase in cardiac metabolism resulting from nerve stimulation are regarded as the primary effects which indirectly give rise to coronary vasodilatation by 1, increasing locally the production and elaboration of metabolites and/or 2, by creating a local relative anoxia caused by a disproportion between the increased rate of  $O_2$  utilization and the existing coronary blood flow.

The rôle played by the cardiac nerves arising from the stellate ganglia is believed to be one associated with the adaptation of the work output of the heart to the blood flow and pressure requirements of the whole animal and not primarily one of coronary vasomotor adjustment to the requirements of the heart.

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# THE LOCAL NATURE OF ACQUIRED RESISTANCE TO TRAUMA

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It has been demonstrated by Noble (1), by Ungar (2) and others (3) (4) that animals subjected to a nonfatal degree of trauma acquire an "immunity," so that after an interval of time they are able to withstand trauma that would ordinarily be fatal. Noble produced standardized trauma by rolling animals in a drum, after the method of Noble and Collip (5). Ungar employed a technique of measured trauma to the legs of animals by means of a metal rod falling from a given height.

These observations have been taken as evidence of a humoral mechanism of resistance, and Ungar reported that the serum of "immunized" animals had acquired the property of protecting normal animals against trauma when injected prior to the trauma; this has not been confirmed by Tobey and Noble (4) or by Prinzmetal (6).

The availability of a large number of mice that had survived tourniquet shock as a result of therapy (7) permitted us to confirm the presence of increased resistance to a repetition of the trauma at the original site. The tourniquet method was also adapted to the front legs of mice so that it became possible to test the effect of reapplication of trauma to a different site upon the development of resistance.

The technique of standardized tourniquet shock in mice has been previously described (7). Our experiments differ from those previously reported on this subject in the animals employed (mice), in the use of tourniquets to produce trauma, and in a more severe degree of initial trauma. It is believed, however, that the nature of the phenomenon is essentially the same under these conditions.

Tourniquets were originally applied to both front or both hind legs for a period of two hours in all but one experiment. In 191 untreated mice in this series this application to the hind legs resulted in an acute mortality of 96 per cent, and in 32 mice with application to the front legs the mortality was 85 per cent. Care must be exercised that the tourniquets on the forelegs do not extend too far anteriorly on the body so as to interfere with respiration by pressure on the trachea. To this end the legs are extended laterally while the tourniquets are being applied. In other respects the technique for front leg application was the same as that previously described for the hind legs.

Groups of mice received oral or intraperitoneal therapy with 8 to 10 per cent of body weight of 0.9 per cent sodium chloride on removal of the tourniquets. This therapy reduces the mortality to approximately 15 per cent for hind leg application (7), and following tourniquets to the forelegs in a series of 65 mice that received saline, the mortality was 14 per cent.

The survivors of these applications to front or hind legs were kept for intervals of 4 to 38 days, after which tourniquets were reapplied for 2 hours to the hind legs of all mice. No therapy was given at this time. The presence of "immunity" in these mice was demonstrated by comparing the mortality from this second tourniquet application to that which occurred in mice from the same batch receiving tourniquets for the first time. These control animals received their tourniquets either at the time of the original application or at the time of reapplication to the other groups.

When tourniquets were originally applied to the hind legs and reapplied after an interval of 21 days a substantial reduction in mortality resulted. In a total of

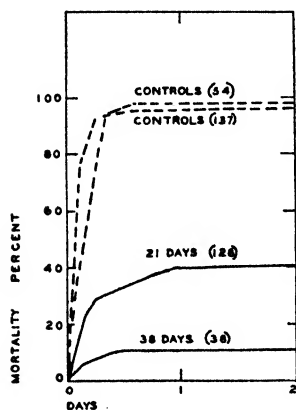


Fig. 1

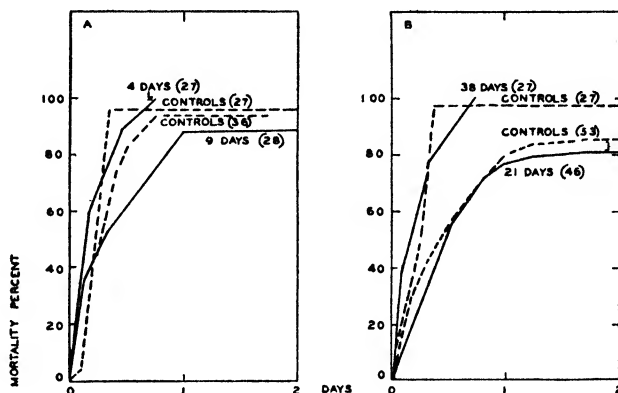


Fig. 2

Fig. 1. Mortality curves following the reapplication of tourniquets to the hind legs of mice that had received tourniquets to the hind legs 21 and 38 days previously (death from the original application prevented by saline therapy). Controls represent untreated mice with tourniquet application for the first time, either at the time of the original (137 mice) or second (54 mice) application to the others. Figures in parentheses indicate number of animals in each group.

Fig. 2. Mortality curves following application of tourniquets to the hind legs of mice that had received tourniquets to the front legs 4 to 38 days previously. Controls represent tourniquets to hind legs in mice without previous tourniquet application. Figures in parentheses indicate numbers of animals used.

128 mice comprising 9 experiments the mortality was 40.5 per cent as compared with 95.5 to 97.5 per cent among 191 controls (fig. 1).

Shorter intervals than 21 days were not satisfactory for reapplication to the same legs because the tissues were too soft to support the tourniquets. After 2 weeks the legs approached their normal size, and at 21 days little evidence of anatomical or functional impairment was present. At this time the weight of the legs, reported below, was not significantly different from normal. Histological changes, however, were still present.

Reapplication of the tourniquets to the hind legs after an interval of 38 days brought about a mortality of 10.5 per cent (fig. 1). While this indicates greater resistance at 38 than at 21 days, the tests were not run simultaneously, and thus the results are not entirely comparable.

In contrast to these results the development of resistance was not observed when the original application was made to the forelegs and the second to the hind legs. Time intervals of 4, 9, 21 and 38 days were permitted to elapse. No significant difference in mortality from the controls was observed in any of these experiments (fig. 2).

In the experiment with a 4 day interval the original application of the bands to the fore legs was for a period of 1 hour and the therapy at that time was 3 per cent of body weight of saline. This amount of trauma in 10 untreated controls was fatal to half of them. In all other experiments the applications were made for two hours.

These observations indicate that the observed "immunity" is a local phenomenon. Histological studies were made to determine the condition of the tissues at the time of reapplication and experiments were also carried out to measure the degree of swelling in these tissues as compared to that of normal tissues following tourniquet application.

Pathological studies of the legs were carried out at various intervals after tourniquet application. The inflammatory and necrotic changes seen in the first few days were followed in 7 to 10 days by active proliferative changes in the areas of necrosis. The greatest necrosis was seen in the areas underlying the tourniquets, and often involved the bone and marrow, where it was accompanied by production of endosteal and periosteal callus. Fibroblast and muscle cell proliferation and leucocyte infiltration was still active at 17 days. At 35 days after tourniquet application the muscles showed regenerative changes and considerable fatty infiltration; varying degrees of fatty degeneration of the nerves were present. It is evident that the process of repair is incomplete on the 35th day even though the gross appearance and functional state approached normal.

Studies were carried out to compare the degree of swelling in legs receiving tourniquets for the second time with legs receiving them for the first time.

Mice that had received tourniquets to the hind legs 21 days previously, along with normal mice from the same batch, were given tourniquets to the hind legs for two hours. Two hours after removal of the tourniquets the animals were immersed into a freezing mixture of solid  $\text{CO}_2$  and ether. The hind legs were cut off, and analyses of the wet and dry weights were made upon pooled samples from each group. The technique and validity of the procedure have been described elsewhere (9).

A period of two hours after tourniquet removal was chosen for analyses because it has been found that the major part of the swelling occurred by then (8) (9). This appeared to be the best time to indicate whether a change in rate was present as well as to give an index of total swelling.

The results are shown in table 1. The weight of legs 21 days after tourniquet application (Ib) was not significantly different from normal (IIb). Upon reapplication of tourniquets the amount of swelling ("fluid loss") (Ia) was similar to that present in the legs that received tourniquets for the first time (IIa), even though the mortality has been shown to differ significantly in the two groups.

In experiments reported elsewhere (9) evidence has been submitted that fluid and sodium loss into the traumatized tissues, and liberation of potassium, are

interrelated, and that all three are of significance in shock. We have studied one of these factors, and the results indicate that within the limitations of the methods

TABLE 1

*Wet weight, dry weight, and water content of two hind legs of tourniquet and control mice*

Values are expressed as grams per single mouse (corrected to body weight of 15 grams), although they represent determinations on pooled samples from the indicated number of animals. Experiments A and B were separated by an interval of six months.

	EXPERIMENT A				EXPERIMENT B			
	No. of animals represented	Wet weight	Dry weight	H <sub>2</sub> O content	No. of animals represented	Wet weight	Dry weight	H <sub>2</sub> O content
(I) Previous tourniquet application 21 days prior to experiment								
(a) Reapplication of tourniquet	4	2.05	0.575	1.47	5	1.71	0.506	1.20
	4	1.97	0.568	1.40	5	1.66	0.478	1.18
	2	2.09	0.585	1.50	5	1.67	0.496	1.17
					5	1.68	0.500	1.18
Average.....	10	2.024	0.574	1.45	20	1.679	0.495	1.18
(b) No reapplication of tourniquet ("Controls")	4	1.37	0.530	0.84	5	1.22	0.512	0.71
	4	1.39	0.495	0.89	5	1.21	0.528	0.68
	4	1.38	0.498	0.88	5	1.26	0.488	0.77
					4	1.24	0.488	0.75
Average.....	12	1.378	0.508	0.87	19	1.233	0.504	0.73
H <sub>2</sub> O difference.....				0.58				0.45
(II) No previous tourniquet application								
(a) Tourniquet group	4	1.95	0.600	1.35	5	1.69	0.522	1.17
	4	2.18	0.690	1.49	5	1.82	0.532	1.29
	2	1.98	0.705	1.27	5	1.71	0.532	1.18
					5	1.61	0.540	1.07
Average.....	10	2.047	0.657	1.39	20	1.708	0.532	1.18
(b) Control group	4	1.32	0.528	0.79	5	1.22	0.492	0.73
	4	1.65	0.683	0.97	5	1.22	0.478	0.74
	4	1.37	0.490	0.88	5	1.23	0.490	0.74
					5	1.18	0.458	0.72
Average.....	12	1.443	0.567	0.88	20	1.211	0.480	0.73
H <sub>2</sub> O difference.....				0.51				0.45

employed the phenomenon of acquired resistance cannot be explained on a basis of variation in fluid loss alone. Further study is required to establish the mechanism of the resistance.

## SUMMARY

Standardized fatal trauma in mice was produced by application of tourniquets to the fore or hind legs. Mice that survived this trauma as a result of therapy were resubmitted to tourniquet application after intervals of time.

When the trauma was applied to a different site in from 4 to 38 days no resistance was present.

When the trauma was reapplied to the same site after 21 or 38 days a high degree of resistance was demonstrated.

Analyses of the legs for the amount of swelling revealed no significant difference in the two groups.

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# THE INTESTINO-INTESTINAL INHIBITORY REFLEX: THRESHOLD VARIATIONS, SENSITIZATION AND SUMMATION<sup>1</sup>

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The qualitative features of the reflex inhibition of the intestine which occurs in response to stimuli arising from alterations of pressure within it have been studied in several laboratories. This information has been summarized in a collective review (5). The present study is concerned with 1, the factors that cause variation in the effectiveness of a given distention, in eliciting reflex inhibition of intestinal motility, and 2, the relation of length of intestine distended to the pressure that is required to initiate the reflex.

**METHOD.** Four dogs were prepared, each having one Thiry and one Thiry-Vella loop which were made from adjacent segments of the upper jejunum. All extrinsic nervous pathways were left intact. Motility from an intestinal segment in the form of a Thiry loop (indicator segment) was recorded by the balloon-mercury-manometer method (7) in unanesthetized, unmedicated animals. A measured volume of water was introduced into the balloon-mercury-manometer system by means of a side-arm, and this volume remained unchanged throughout the experiment. Motility in this segment served as an indicator for the effects of pressure changes produced by means of balloons in a Thiry-Vella loop. Pressures in the two balloons used for distending portions of the Thiry-Vella loop were controlled by connecting a gravity pressure bottle with the balloon-mercury-manometer systems. In some studies, balloons of varying length were successively introduced, so as to include the same section of the Thiry-Vella loop. In other experiments, balloons were placed one within the proximal and the other within the distal end of the Thiry-Vella loop, and a distending pressure was established alternately or simultaneously in the two balloons.

**RESULTS.** The observations described are based on 60 experiments in 4 dogs, involving more than 300 distentions.

**I. Variations in effectiveness of a single distention.** Previous studies of the reflex in unanesthetized animals have indicated that intensity, duration and rate of change of the stimulus are factors which alter the reflex response. The response to graded distentions of a segment of intestine is a graded reflex inhibition of the indicator segment. A single distention may be ineffective, subthreshold, threshold, submaximally effective or maximally effective. Further observations confirm the previous report (7) that the threshold for an effective distention is elevated when there is a slow rate of increase in the pressure; and that a sudden increase in pressure is more effective in producing inhibitory effects than a step-by-step increase to the same or even to a somewhat higher level. There is slow adaptation to a distending pressure, and sustained pressures result in prolonged inhibitory effects.

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A distending pressure in one end of a Thiry-Vella loop, when just sufficient to inhibit the opposite end of the segment, may cause no inhibitory effects in the indicator segment which has no intrinsic connections with the loop distended. This result is illustrated in figure 1. This is further evidence that the intrinsic mechanisms of the intestine contribute to the inhibitory influences of intestinal distention.

There is a variability in the minimal pressure that is required to elicit the reflex, in different animals and in the same animal under altered conditions. Youmans, Karstens and Aumann (6) observed that wide individual variations exist

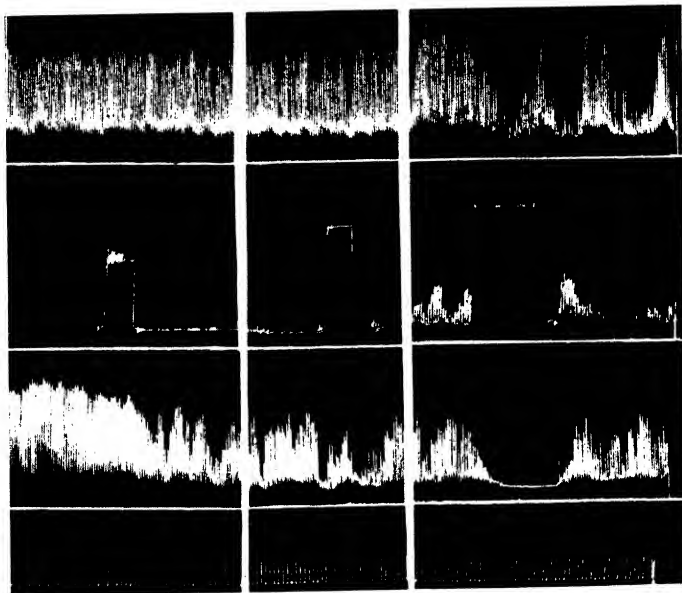


Fig. 1. Comparison of responses in the proximal end of a Thiry-Vella loop with response in a Thiry loop, produced by distention of the distal end of the Thiry-Vella loop. From above downward there is illustrated 1, motility of Thiry loop; 2, zero pressure level; 3, distal end of Thiry-Vella loop; 4, zero pressure line; 5, proximal end of Thiry-Vella loop; 6, zero pressure level; 7, time in 10 second intervals. Breaks in record, duration of 10 and 20 minutes respectively. Distending pressures, 45, 60 and 72 mm. Hg respectively. See text for further explanation.

between animals. Pressures from 35 to 100 mm. Hg. were required to elicit the reflex. In the present study, the pressure required to elicit comparable inhibitory effects in the same animal, on different days, has varied not more than 8 mm. Hg. Theoretically, it might be expected that a distention in one part of the jejunum would have a different reflex effect than an identical distention in a more distal part. However, comparable submaximal inhibitory effects were obtained in the indicator segment from identical distending pressures in the same balloon, placed first in the distal then in the proximal end of the Thiry-Vella loop. The loops were 30 cm. long. The possibility remains that a gradient effect might be demonstrated, by comparing reflex effects of distention of two intestinal segments that are more widely separated than those used in the present study.

II. *Sensitization by a previous distention.* Lalich, Herrin and Meek (3) reported that the intestino-gastric inhibitory reflex is reinforced by subjecting a Thiry loop to a continuous distending pressure for twenty-four hours before redistention. It has been shown that after a previous distention of the jejunum, if the rate of change of the stimulus is not altered, a second distention of shorter duration will surpass the first in causing inhibitory effects in an indicator segment (7). No attempt was made to determine whether the reinforcement was on the basis of a central or a peripheral sensitization. In the present study, it was found that the threshold for the intestino-intestinal reflex could be reduced by as much as one-half, by a series of brief, effective distentions. The successive distention of the same section of a Thiry-Vella loop with subthreshold pressures will finally produce reflex inhibitory effects in the indicator segment. If a single

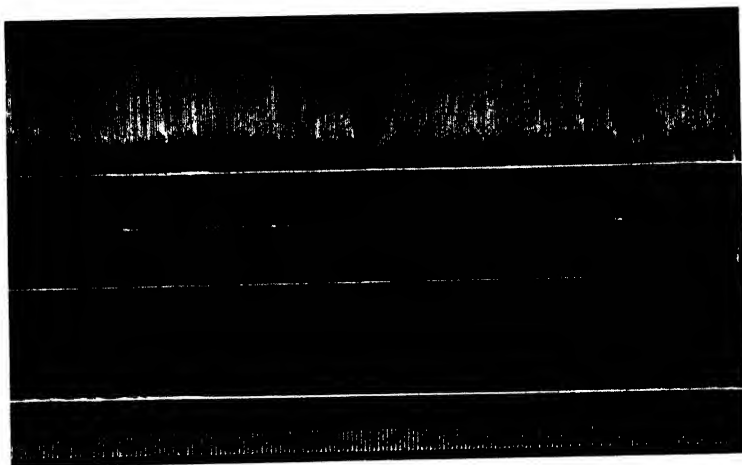


Fig. 2. Sensitization of the intestino-intestinal inhibitory reflex by a previous distention, and demonstration that sensitization is peripheral and at the site of distention. From above downward there is shown 1, balloon-mercury-manometer record of jejunal Thiry loop (indicator segment) in an unmedicated, unanesthetized dog; 2, zero pressure level; 3, pressure record in proximal end of a Thiry-Vella loop, distending pressure of 26 mm. Hg in a 5 cm. balloon; 4, zero pressure level; 5, pressure record in distal end of the Thiry-Vella loop, distending pressure of 26 mm. Hg in a 5 cm. balloon; 6, zero pressure level; 7, time in 10 second intervals. See text for further explanation.

subthreshold pressure is maintained for a prolonged period of time, it may so sensitize the reflex mechanism that redistention will result in inhibition of the indicator segment. This result is illustrated in figure 2.

Possible sites in the reflex arc at which sensitization might occur are at receptor end organs, at synapses or at the neuro-effector junction. The following results indicate that the sensitization is peripheral and at the site of distention. If the threshold effective pressure is determined for a section of the Thiry-Vella loop, distention of a second section of the segment with a pressure which would ordinarily sensitize that area to redistention does not influence the threshold for the reflex in the first section. A sustained and prolonged *subthreshold* distention in one end of the Thiry-Vella loop will sensitize locally, so that a subsequent distention of similar intensity at the same site will elicit the reflex. However, a sub-

sequent identical distention of the opposite end of the segment is ineffective. This result is illustrated in figure 2.

The exact nature of the peripheral sensitization has not been determined. The alteration in irritability of receptors is roughly proportional to the intensity and duration of the distending pressure. Sensitization to redistention occurred when a distending pressure of 26 mm. Hg. was used. This is illustrated in figure 2. Thus sensitization occurs at pressures significantly lower than those required to block the local circulation (2) and well within the range of pressure created by intestinal contractions. This result introduces the possibility that the sensitization observed in these experiments may be a physiologic phenomenon.

III. *Relation of length of intestine distended to pressure required to elicit the reflex.* The minimal pressure which will elicit the intestino-intestinal inhibitory reflex is comparatively high, when a distending balloon 5 cm. in length is used. Pressures ranging from 35 to 100 mm. Hg. are required (6). Such pressures far exceed intra-enteric pressures recorded from clinical cases of intestinal obstruction (4), though they are approximated by the pressures developed in the closed-loops of experimentally obstructed animals (1). Previous studies do not indicate whether the intestino-intestinal reflex functions within physiologic limits, or if it can be elicited only by high, artificially-produced, distending pressures. It is on this basis that one might question the rôle of reflex inhibition of intestinal motility by intestinal distention as a contributing mechanism to the perpetuation of distention, in ileus and intestinal obstruction. However, the minimal pressure that is required to elicit the reflex when a 5 cm. section of bowel is distended provides no information concerning the fundamental problem of the minimal intraluminal pressure that will initiate impulses on the afferent side of the reflex arc.

The rôle of spatial summation in the production of a normal reflex discharge indicates that this phenomenon would be expected to occur, when the intestino-intestinal reflex is activated. It can be shown in several ways that summation does occur. The results of one type of experiment demonstrating summation are illustrated in figure 3. The simultaneous combination of *subthreshold* distentions in two distending balloons of equal length, placed in opposite ends of a Thiry-Vella loop, is followed by inhibition of tonus and motility of the indicator segment. Control distentions, before and after simultaneous combination of the distending pressures, offer evidence that sensitization by previous distention of either segment is not responsible for the inhibition.

Another type of experiment demonstrating summation is illustrated in figure 4. The proximal end of a Thiry-Vella loop was first distended by a pressure of sufficient magnitude to elicit severe inhibition when a 15 cm. segment of the Thiry-Vella loop was utilized. An identical distending pressure was less effective after the balloon had been changed, so as to involve only a 5 cm. segment. The possibility that the results of this experiment may be explained by sensitization, or by differences in the threshold on the basis of an intestinal gradient, is ruled out by the following facts. The distention of the shorter section is *less effective* following a previous distention in the longer section of the segment with an identi-

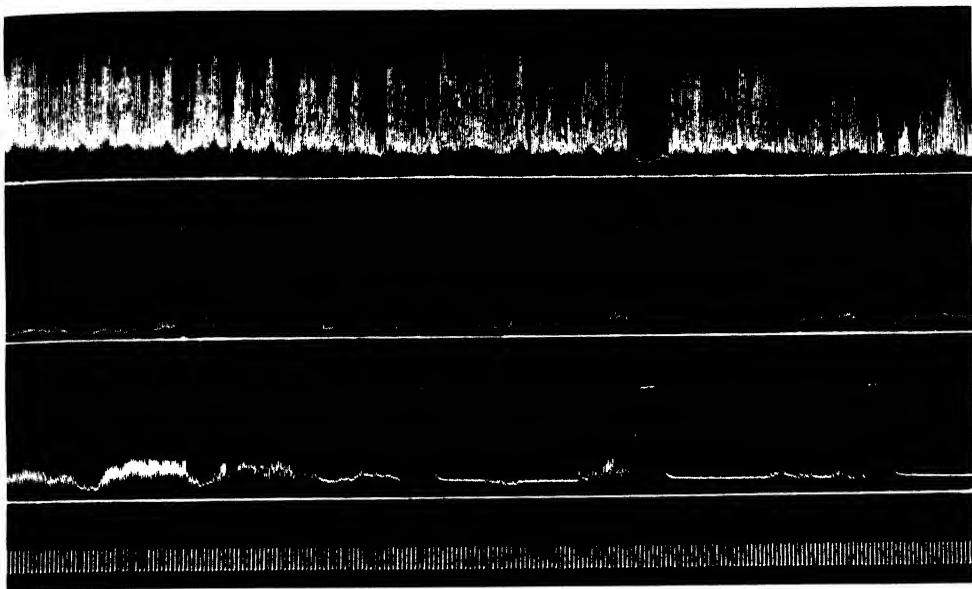


Fig. 3. Summation of the intestino-intestinal inhibitory reflex, shown by inhibitory effects on the indicator segment from the combination of separately subthreshold distentions of the proximal and distal ends of a Thiry-Vella loop. From above downward the writing points are arranged as in figure 2. See text for further explanation.

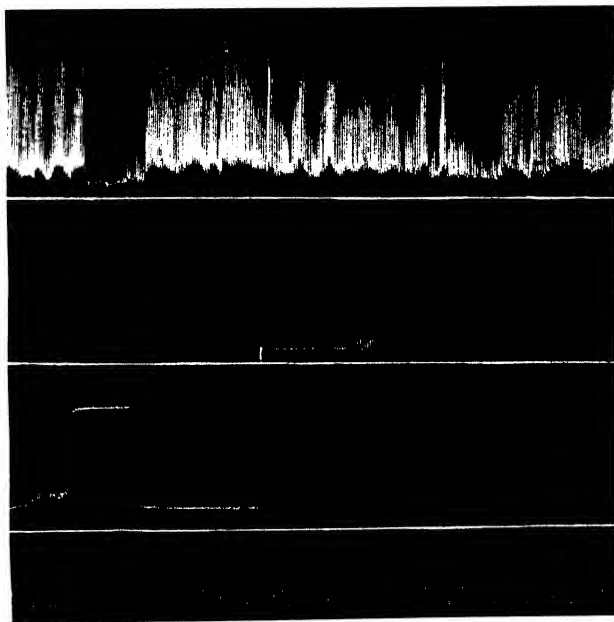


Fig. 4. Relation of length of intestine distended to pressure required to elicit the intestino-intestinal inhibitory reflex. Summation of reflex effects, as evidenced by a lowering of the distending pressure required to elicit the reflex as the length of the segment distended is increased. Upper record from indicator segment. First distention, 15 cm. balloon in proximal end of a Thiry-Vella loop. Second distention, identical pressure in a 5 cm. balloon placed in the proximal end of the Thiry-Vella loop. Time in 10 second intervals. Balloon changed, record stopped for 3 minutes, at break in record. See text for further explanation.

cal pressure, in spite of the fact that sensitization would tend to make the second distention *more effective*, if the balloon length were unchanged. The results presented in section I indicate that gradient effects do not require consideration in these experiments, where the balloons are not more than 20 cm. apart. Moreover, adjacent segments of intestine were distended in these experiments.

#### SUMMARY AND CONCLUSIONS

1. The factors that determine the minimal pressures required to elicit the intestino-intestinal inhibitory reflex have been studied in unanesthetized dogs, by recording the responses of one intestinal segment (Thiry loop) during the distention of another segment (Thiry-Vella loop) with balloons of various lengths and utilizing various pressures.

2. The minimal pressure required to elicit the reflex is lowered as the length of the jejunum distended is increased. The effectiveness of a given pressure in eliciting the reflex is greater as the length of the jejunum distended is increased. These results may be readily explained on the basis of spatial summation in the central nervous system, or in the autonomic ganglia involved.

3. An effective distention of any duration, or a prolonged subthreshold distention, will sensitize the intestinal segment to redistention. Evidence is presented that this is a peripheral sensitization occurring at the site of distention.

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# EVIDENCE OF THE PERIPHERAL ACTION OF VITAMIN D FROM X-RAY DIFFRACTION STUDIES<sup>1</sup>

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In recent publications (1, 2) it has been shown that the physiological orientation present in the cortex of the long bones of rats may be disorganized in a rachitic state, and that this condition is not repaired rapidly on a healing regimen. Also, it was shown by breaking tests that disorientation is not closely correlated with decreased resistance to lateral breaking stress and that the latter function is restored before complete reorientation.

Subsequent studies, with improved technic to be published in detail later, have shown that the disorientation is much more severe than was recognized at first or has been reported by other observers. Instead of confinement to two planes indicated by the 5 inch and 8 inch rings in our type pattern diffractogram (1) of apatite and bone powder (2) the disturbance is manifested also in all planes that show any orientation in healthy bone. In table 1 are listed spacings and indices showing a total of 5 planes subject to orientation, disorientation and reorientation by metabolic processes.

Hendricks and Hill (3) published a formula for bone salt together with those derived by others. Our findings, also to be reported in detail elsewhere, do not confirm their formula but do tend to confirm more closely that given by Klement (4),  $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ , which is essentially the formula generally accepted for apatite.

The technical procedures of diffraction work have been described (2). The present experiment was undertaken in an effort to determine the influences involved in reorientation.

The basal diet consisted of Fox Chow Meal (Purina) supplemented with fresh lettuce twice weekly. Different lots of this have been analysed repeatedly for protein, Ca, P and caloric value. These analyses do not differ importantly from the data declared by the manufacturers. The mean caloric value of eleven determinations by bomb calorimeter was 500 cal. per 100 grams protein, 23 per cent; calcium, 1 per cent; phosphorus, 0.83 per cent.

This diet has proven adequate for the age period in the life span of the rat covered by these experiments. Over a period of seven years there has been no indication of any nutritional deficiency on this diet and the growth performance has far exceeded that recorded for most rat colonies.

Three groups of rats were made up at weaning time as follows:

IC. Controls, 14 males, 5 females. These were kept on the basal diet throughout. Beginning at 85 days at intervals, one rat was killed and all bones re-

<sup>1</sup> Supported in part by grants from the Graduate School Research Board, the Ella Sachs Plotz Foundation and the Nutrition Research Laboratories.

covered, up to 220 days when the group was exhausted. Diffractograms were made from the cortices of the tibial midshafts. All of them showed a high degree of orientation in all planes indicated in table 1.

IHR. Fourteen rats, 8 males and 6 females. At 21 days of age these rats were placed on the Steenbock rachitogenic diet no. 2965 for 55 days, at which time one rat was killed and the rest restored to the basal diet. At intervals up to 175 days one animal was killed and the bones examined as above. Not until 39 days on the basal diet was a good degree of reorientation found.

IDR. Fourteen rats, 8 males and 6 females. These were treated similarly except that at 55 days, instead of being restored to the basal diet, they were continued on the rachitogenic diet with a daily supplement of 75 units of vitamin D in oil, by mouth. The last rat was killed after 171 days of this treatment. By all accepted criteria of antirachitic healing all rats in both groups were healed after 6 weeks although growth rates were not restored in group IDR.

TABLE 1

RING NO.	$\frac{d}{A}$	INDICES	ORIENTATION
3	4.90	{ 1120 2020	None
4	3.85	1121	None
5	3.50	2021	Marked
6	3.22	1012	None
7	2.84	2131	None
8	2.67	3030	Marked
9	2.30	2132	None
10	2.00	1123	Marked
11	1.85	3250	None
12	1.63	3252	Marked
13	1.58	3143	Marked

Since it is difficult to characterize degrees of orientation mathematically so as to permit statistical treatment, we adopted three grades of orientation, *good*, the maximum degree of orientation, *fair*, distinctly less, and *poor*, closely resembling bone powder diffractograms, or those of powdered apatite, in which there is no orientation.

After omitting data for the animals in each group killed before rachitic healing was complete, in all of which poor or only fair reorientation was found, the following comparison may be made among ten rats in each group. Of the IHR group 4 (40 per cent) showed poor orientation, 3 (30 per cent) fair, and 3 (30 per cent) good. In the IDR group there were 3 (30 per cent) poor, 4 (40 per cent) fair, and 3 (30 per cent) good. It is obvious that the data are too limited to permit any conclusion other than that the rachitogenic diet plus vitamin D reorients as readily as the basal diet although it does not restore growth rates.

Since the results were not as clear as is desirable in these preliminary experiments the procedure was repeated with larger groups of animals. Also both the duration of the rachitic period and the restorative period were varied.

Group IIC. The growth curves of these animals are not remarkable and therefore are omitted but are available for examination if desired.

There were 29 rats, 12 females and 17 males. The age at death ranged from 43 to 226 days. Examination of the diffractograms of the cortices of the tibial midshafts shows that there was a high degree of orientation in all but 4 animals. One of the latter showed complete disorientation. The other 3 showed moderate degrees of orientation but were easily recognizable as deficient. One of these was a female, the other 3 were males. These were, respectively, 146, 160, 189 and 202 days old.

These 4 animals demonstrate the sensitivity of this reaction to other metabolic disturbances as already mentioned (2). While none of the 4 showed any gross evidence of metabolic disturbance yet there must have been some process that either disturbed normal alignment or prevented proper development that was independent of the diet *per se*, since the basal diet has been found to be an adequate regimen for more than 3000 rats in our colony. This point has an important bearing on later discussion.

Group II HR (table 2) comprised 35 animals, 15 females and 20 males that were placed on the Steenbock rachitogenic diet no. 2965 for periods ranging from 50 to 80 days and then returned to the basal diet for periods up to 125 days. In the table the column headed "R days" indicates the number of days on the rachitogenic diet, the one headed "H days", the time on the healing diet. No correlation could be established between the degree of orientation found at death and the total age, or the "R days". In fact, evidence in another experiment shows that a simple Ca-deficient diet may produce marked disorientation in 10 to 15 days.

However when the data are arranged in ascending order of the number of "H days", it is possible to make a partial correlation. It must be recalled that by the usual diagnostic criteria of rickets, such as the line test, breaking stress and roentgenographic films, the rachitic state disappears usually long before reorientation is complete.

Since it appears that reorientation, in the more severe degrees of disturbance, is not much in evidence before 6 weeks of healing treatment, the group was divided into those killed within 42 days on the basal diet, 11 in number, and those killed after that time, 24 in number. Again there was no correlation between the degree of orientation on the one hand and the number of R days, or the total age on the other. Out of the 11 animals, 8 (72.7 per cent) showed very marked disorientation and 3 (27.7 per cent) moderate degrees. In none was there complete reorientation. Of the 24 animals surviving more than 42 "H" days, 11 (45.8 per cent) showed complete reorientation, 6 (25 per cent) moderate degrees, and 7 (29.2 per cent) very poor reorientation. The total ages of these animals at the time of death ranged from 92 to 216 days.

The question at once arises, why should there be any disturbance of orientation after 42 days on the basal diet? The answer must be speculative in part. If it is accepted that the rachitic state is a systemic, metabolic disturbance, then it is not difficult to envision a residuum of this disturbance after healing



treatment has been instituted that would vary in magnitude. Therefore, the rate of reorientation may vary greatly. It has been shown that there may be some persistence after 275 days of antirachitic treatment (2).

Besides, the possibility of undetected metabolic influences, as stressed above, cannot be overlooked here. With a metabolic complex already upset it may be that a diet calculated to be adequate may actually be physiologically deficient in the sense that the organism cannot make the most efficient use of it. However the possibility that the oriented state of bone crystals has a metabolic significance wholly unrelated to known metabolic processes must not be over-

TABLE 2  
Group II, HR

NO.	SEX	DAYS			ORIENTA- TION	NO.	SEX	DAYS			ORIENTA- TION
		R	H	Total				R	H	Total	
1	M	75	0	96	Poor	19	F	69	63	153	Good
2	F	75	0	96	Poor	20	M	68	63	152	Good
3	M	74	7	102	Poor	21	M	68	70	159	Good
4	F	74	7	102	Poor	22	M	68	70	159	Poor
5	F	74	14	109	Poor	23	F	68	77	166	Poor
6	M	54	24	104	Poor	24	F	68	84	173	Good
7	M	50	24	104	Fair	25	F	68	84	173	Fair
8	F	56	25	94	Poor	26	M	66	91	177	Poor
9	M	56	25	94	Fair	27	F	66	91	177	Fair
10	F	54	35	111	Fair	28	M	68	98	187	Poor
11	F	54	35	111	Poor	29	M	68	98	187	Good
12	F	70	52	143	Fair	30	M	68	108	187	Fair
13	F	70	52	143	Fair	31	M	68	105	194	Good
14	M	69	56	157	Good	32	M	71	112	213	Good
15	M	69	56	146	Good	33	F	70	119	210	Poor
16	M	70	59	150	Poor	34	F	70	119	210	Fair
17	M	70	59	150	Poor	35	M	69	125	216	Good
18	M	68	63	152	Good						

looked. Regarded in this light, it is remarkable that so little disturbance was found, even in the longer periods of deficient nutrition.

In an effort to evaluate more fully the possible interrelation of "R" time and "H" time, several statistical procedures were adopted, none of which added materially to the information obtainable from simple inspection of table 2, namely, that the degree of reorientation is a function of healing tie. The tables derived are omitted to conserve space.

Group II DR (table 3) comprised 20 female and 10 male rats placed on the rachitogenic diet (Steenbock no. 2965) for periods ranging from 50 to 80 days and then instead of restoration to the healing diet as in the HR group, each animal received a daily supplement to the rachitogenic diet in the form of 75 units of vitamin D in oil, by mouth. Two animals were killed at this time and the others at periods up to 126 days. The age at death was from 74 to 217 days.

Again it appears that no significant reorientation occurs before 62 days of treatment, but there was no other direct correlation with the "R" days nor the total age in days.

Again various statistical procedures were employed in an effort to evaluate the results but without adding materially to that obtainable by inspection of the table. Good reorientation was preponderant beyond 42 days of healing ("D" days in table 3). There seems to be as good reorientation induced by the vitamin D alone added to the rachitogenic diet as by the basal diet.

Furthermore, it seems to be strongly indicated that vitamin D exerts a function in promoting orientation in opposition to other numerous factors that can disarrange the crystal arrangement of bone. It is evidently not able, in the amounts used here, to combat completely all of the disorganizing influences.

TABLE 3  
*Group III, DR*

NO.	SEX	DAYS			ORIENTA- TION	NO.	SEX	DAYS			ORIENTA- TION
		R	D	Total				R	D	Total	
1	M	74	0	96	Poor	16	M	70	49	140	Good
2	M	74	0	96	Poor	17	M	52	49	122	Good
3	M	52	1	74	Poor	18	F	56	56	132	Good
4	M	52	4	77	Poor	19	F	52	60	133	Good
5	F	74	7	103	Poor	20	F	52	60	137	Good
6	F	74	7	102	Poor	21	F	54	69	145	Good
7	F	52	11	84	Fair	22	M	52	69	143	Good
8	F	74	14	109	Poor	23	F	68	70	159	Good
9	M	56	21	94	Fair	24	M	68	70	159	Good
10	F	56	21	94	Good	25	F	55	70	146	Poor
11	F	54	28	104	Poor	26	F	55	70	146	Fair
12	F	54	35	110	Poor	27	F	52	81	154	Good
13	F	54	35	110	Poor	28	M	66	91	177	Good
14	F	56	42	115	Good	29	F	66	91	177	Poor
15	F	55	45	121	Fair	30	F	70	126	217	Good

If this disorganization is a fundamental factor in rickets then the physical structure of bone is evidently not only more sensitive to injury but more resistant to repair than other changes accepted as diagnostic criteria of rickets. This renders the rôle of vitamin D all the more significant since, taken alone, it is able to repair the particular disturbances as adequately as the basal diet.

For a generation, it has been contended that vitamin D promotes healing of rickets by improving the absorption of calcium or phosphorus, or both. This has always seemed to us a superficial interpretation of the facts. On the other hand, the proponents of alternative theories have probably been equally superficial in interpreting data.

No satisfactory mechanism has ever been suggested to explain the alleged improved absorption. It is not claimed that the experiments reported here offer any decisive evidence against the theory of improved absorption. They

do make clear that vitamin D exerts a peripheral effect on the physical structure of bone. To what extent disorientation is fundamentally involved in the production of rickets is yet to be determined.

If antirachitic healing depends solely on increased absorption of bone minerals, then the parenteral administration of the minerals should promote healing of rickets. So far as we are aware, no one has successfully treated experimental rickets for any length of time by parenteral administration of Ca and P. We have several times attempted this, intraperitoneally in rats, and intravenously in puppies. The technical difficulties were never sufficiently well overcome to produce an entirely clear cut experiment, but the fact remains that never at any time did buffered solutions administered by either route produce the slightest evidence of healing. Others have claimed some degree of success for short periods.

The present experiments, then, seem to indicate that in one respect, at least, vitamin D exerts a local action in osseous tissue in the repair of a non-specific physical disturbance of bone. It is admitted that the evidence could be more conclusive. However, whatever positive support is derived is all the more significant when one considers the technical difficulties inherent in such an experiment. The mechanism of such an action must be catalytic but no definite mechanism can be considered as of proven significance. The recent work of von Kraemer and Landtman (5) suggests that the *amount* of ossification depends on stimulation of the osteoblasts by vitamin D. The evidence for this suggestion seems clear. However, the uptake of Ca by bone, if dependent on osteoblastic activity, would be related mainly to areas that are not normally oriented at all, namely, the epiphyses and the spongy portions along the medullary canal. Consequently, it seems unlikely that the phenonema we have described are particularly related to osteoblastic activity. And yet, the only other mechanism would be a catalytic force capable of changing the physical contiguity of unit crystals.

There was no close correlation between retardation of growth rates and degrees of physical disorientation, but the curves have been omitted to conserve space.

The apatite type of molecule does not form in the body except in bone. Its synthesis may depend on some factor other than vitamin D. Its arrangement in the bone may depend partly on vitamin D.

#### CONCLUSIONS

1. Experiments are described in which rats were placed on a rachitogenic diet until severe rickets was present. Then one group was placed on the basal diet, the other receiving a supplement of vitamin D but no other modification.

2. Diffractograms of the cortices of tibial shafts showed that in rickets the disorientation of the crystal pattern was severe but that it had begun to reorient by the time antirachitic healing was complete. Subsequently the completeness of reorientation seemed to be independent of the ordinary evidences of antirachitic healing.

3. Vitamin D added to the antirachitic diet produces as good reorientation

as the basal diet, but does not restore the growth rate. Therefore, reorientation is not due to somatic growth per se.

4. These data indicate a peripheral catalytic action of vitamin D in osseous tissues.

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# THE INFLUENCE OF AGING IN MAN UPON HIS CAPACITY FOR PHYSICAL WORK AND UPON HIS CARDIO-VASCULAR RESPONSES TO EXERCISE

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The attention of biologists (using this term broadly) has been, by this time, fully aroused in respect to the problems of aging. The literature on gerontology and kindred subjects is becoming more and more abundant, and when the Soviet material has been made available through translation, our knowledge will be increased considerably more.

An important question, one which is especially in need of more extensive study, concerns the limits of the physical work which can be expected from persons of advancing years.

There are of course many observations, some of great value, which have been made already. For example, ballet-masters have sometimes very clearly in mind the age-capacity curve of their dancers. These and similar observations wait to be collected, classified and appraised. But all such material must be corroborated and greatly extended by ergometric experiments before the work-physiologist can feel a reasonable degree of security and satisfaction.

There are two ways of conducting an inquiry into the effect of such long continued influences as aging. These are first, the study of the reaction of different age groups, and secondly, the study of the life histories of individuals. Each of these methods has its own peculiar dangers and defects but those of the first, the statistical method, do not concern us at this time, for in the experiments about to be described only the second, the case history method, has been used. This has a conspicuous defect in the difficulty of obtaining suitable material. Age physiology is a relatively new interest. If, therefore, we are to make our discussions cover many years, we must be content with data gathered by methods now out of date and gathered without reference to the special demands of the age-physiologist. Thus certain observations, omitted in 1914 as irrelevant, would, if they had been made, be regarded by the writers of this article as most welcome additions.

Whenever our task is to measure maximum physical capacity, as in the experiments about to be described, it is absolutely necessary that the subject be willing to do his best. He must not be deterred from effort by timidity, nor laziness, nor dislike of discomfort, which often operate almost unconsciously. He must have the "will to work."

A striking instance of the influence of the will to work has been observed by one of us (F. A. H.). In looking over the records of the women riders of the cycle-ergometer reported by McCrea et al. (4), F. A. H. was impressed by the

feebleness of their performance. For, although these six subjects were 20 to 30 years of age and were physical education majors, they performed only 6,480 kg.m. in 10 minutes, an average rate of 648 kg.m./min.

Thinking that these riders might have lacked the will to work, F.A.H. in 1942 secured for a subject P. V. K., a physical education major, age 20, who had great enthusiasm for physiological experimentation and who was willing to exert herself to the utmost. P. V. K. performed 21,000 kg.m. in 30 minutes, at an average rate of 700 kg.m. per minute. Then a second subject, much less robust and enthusiastic, was induced to ride at the same pace. This she kept up for 25 minutes only, but declared that, had she had sufficient sleep the night before, she could have held out for the other five minutes. Then other students were called in, and these, following the example of their predecessors made high records.

In the experiments of Robinson (5), the small boys and the aged men differed from the young adults in many particulars. But were the differences due to age or to the fact that the young adults approached the effort with a "bolder and more competitive attitude" (Robinson's phrase) and were willing to face more discomfort? The question is left undecided. Such results strongly emphasize the difficulty in experiments involving maximal effort. In the case of age studies there is perhaps an additional complication, for it may be that the will to work is so closely related to age as to render age groups incomparable for purely physiological studies! Doubtless the unwillingness to work in the old and in the very young is biological and protective. But in the case of the old at least the inclination toward self protection is re-enforced by social pressure. Thus the person of advancing age is likely to be dissuaded from maximal effort by the anxiety of his friends and to assume a timid attitude which is a caricature of prudence.

One should not, however, entertain the notion that by maximal effort we mean such devotion as was exhibited by the runner of the *first* marathon. We mean an effort which is well borne, which results in no untoward signs or symptoms, but is as productive as possible without so doing.

**EXPERIMENTS.** *The subject.* The subject of these experiments has never been exceptionally endowed physically. Indeed his clinical history shows an unusually large number of medical and surgical ailments. But all of these troubles have had in common this outstanding characteristic, namely, complete and rapid recovery. His athletic history is life-long: hiking for nature study as a boy; intercollegiate competition as a student; and walking, mountain climbing and exacting experiments on the cycle-ergometer in later life. He has never used tobacco in any form, alcohol very rarely and coffee only occasionally. His "will to work" has not so far been questioned.

*Method.* The subject rode a "bi"- cycle-ergometer provided with a cyclometer and adjustable brake either mechanical or electrical. Arterial pressures were determined by auscultation; the beginning of the fourth phase being regarded as representing diastolic pressure. The rides were performed at the following ages, 41, 53, 57, 68 and 71 years. For convenience they will be designated

41, 53, 57, 68 and 71 respectively. Of these 41 (2) and 53 (4) have already been reported in this Journal and 68 has received a preliminary notice elsewhere (3).

The *immediate* preparation for the riding tests which are about to be compared is as follows: 41, 3-mile run performed on 13 occasions; 53, a series of considerable road walks (30-40 miles) in rolling country; 57, a series of 21 rides on the cycle-ergometer; 68, a couple of months in the White Mountains of New Hampshire with frequent ascents chiefly in the Presidential Range, followed 3 weeks later by 2 rides on the cycle-ergometer; 71, a series of 17 rides on the cycle-ergometer. We shall see that the preparation at 53 and 68 was probably inadequate especially in the case of 68.

*Working capacity.* It is unfortunate that the rides at 41 are not strictly comparable with those performed later. The latter were continued for exactly 30 minutes, while the rides at 41 did not last so long. The ride which has been selected as typical of this series (41) was made at top speed but lasted only 18 minutes. During this ride the work done was 38,654 kg.m. If the subject had continued at the same speed for 30 minutes, he would have done 64,423 kg.m. of work. But he did not do so, nor could he have done it, and so it devolves upon us to assign a probable value. For this, the experiments described in this article give us no clue, but from the results obtained from a study of mountain ascents (to be published elsewhere), it seems that 55,000 would be a reasonable figure and the latter appears, therefore, in the accompanying table.

In this table, 53 is the single ride recorded by McCrea et al. (4); 57 av. is the mean performance during the latter part of a series of 33 rides, i.e., the curve of learning was excluded from the calculation; 57 max. is the best and 30th ride of the same series, namely, that of February 22; 68 is the ride of October 29, the maximum of its series and the third from the beginning of the series; 71 is the ride of August 18, the maximum of its series and the 22nd from the beginning.

The low records at 53 and 68 are probably to be accounted for by the inadequacy of the preparation. On both occasions the subject was in good "condition." At 53 he had been walking extensively; at 68 mountain climbing. But at 53 the test ride was the only ride and at 68 it was only the third ride. That the low score at 68 was not due to a lack of the "will to work" is shown by the untoward accompaniments of the test ride (Oct. 29). For toward the end of the ride there was excessive dyspnea, some gastric regurgitation and the subject finished in great distress. On leaving the laboratory an hour later, he was attacked by abdominal pains which disappeared only after twenty minutes' walking in the street.

It seems therefore plausible to conclude that neither walking long distances nor many hours of mountain climbing afford an adequate preparation for the cycle-ergometer ride although, judging from the experience at 41, the 3-mile run is adequate.

It may be of interest to note in this connection that, after the rides at 71 had been completed (22 rides in all), the subject went climbing in the White Mountains and found this new form of exercise very arduous indeed for the first few weeks. So as a preparation for mountain climbing the cycle-ergometer is as

inadequate as is mountain climbing or walking for the cycle-ergometer. All of which is not at all surprising when viewed in retrospect.

Other factors may have contributed in increasing the difficulties at 53 and 68. At 53 the subject wore an x-ray plate and holder strapped to his chest and at 68 he was being tortured by an uncomfortable saddle.

*Circulatory reaction to exercise.* At 41 arterial pressures were determined before, after and during the rides. The same is true at 57, 68 and 71, and in addition the pulse rate was determined at the last three and at 53. Throughout the following discussion it is to be borne in mind that we are not dealing with reactions to a fixed task but with reactions called forth by maximal effort. This difference was not made clear enough in a previous publication (2), a fact which seems to have led to some misunderstanding (1).

In discussing the circulatory reaction to exercise, we turn to figure 1. Here are shown four curves of pulse rate: 53 comprises only four determinations made during a single ride; 57 is the average of eight afternoon single rides (as

TABLE 1  
*Work capacity in relation to age*

AGE	TOTAL WORK	WORK PER MIN.	DURATION	RELATIVE PERFORMANCE			
				%	%	%	%
	<i>kg.m.</i>	<i>kg.m.</i>	<i>min.</i>				
41	38,654	2,147	18				
41	(64,423)	(2,147)	(30)	(100)			
41	55,000	1,830	30		100		
53	31,505	1,051	30	49	57		
57 av.	32,682	1,089	30	50	59	100	
57 max.	33,074	1,102	30	51	60		100
68	24,490	850	30	39	44	78	75
71	28,600	953	30	45	52	87	86

contrasted with "double rides", v.i.); 68 is the average of all single rides in which 21,000 kg.m. or more were performed, namely, 4 rides out of 5; 71 is the average of all rides in which 26,000 kg.m. or more were performed, namely, 6 out of 22.

Here 57 and 71 are very much alike, but 53 and 68 run high during the latter part of the ride. And when for these average values we substitute those found in the particular experiments in which the work done was maximal, namely, February 22, October 29 and August 18, the picture is unchanged for during the latter part of the ride 68 still lies well above 57 and 71, the latter being the lowest of the three.

The high position of 53 and 68 are perhaps due to the inadequate preparation already discussed.

In the same figure are shown four sets of arterial pressures: 41 is experiment of May 29 (2); 57 is February 22 of which the diastolic pressure is somewhat above the average in its series; 68 is October 29; and 71 is August 18. In other words all are individual rides each the maximal of its series.



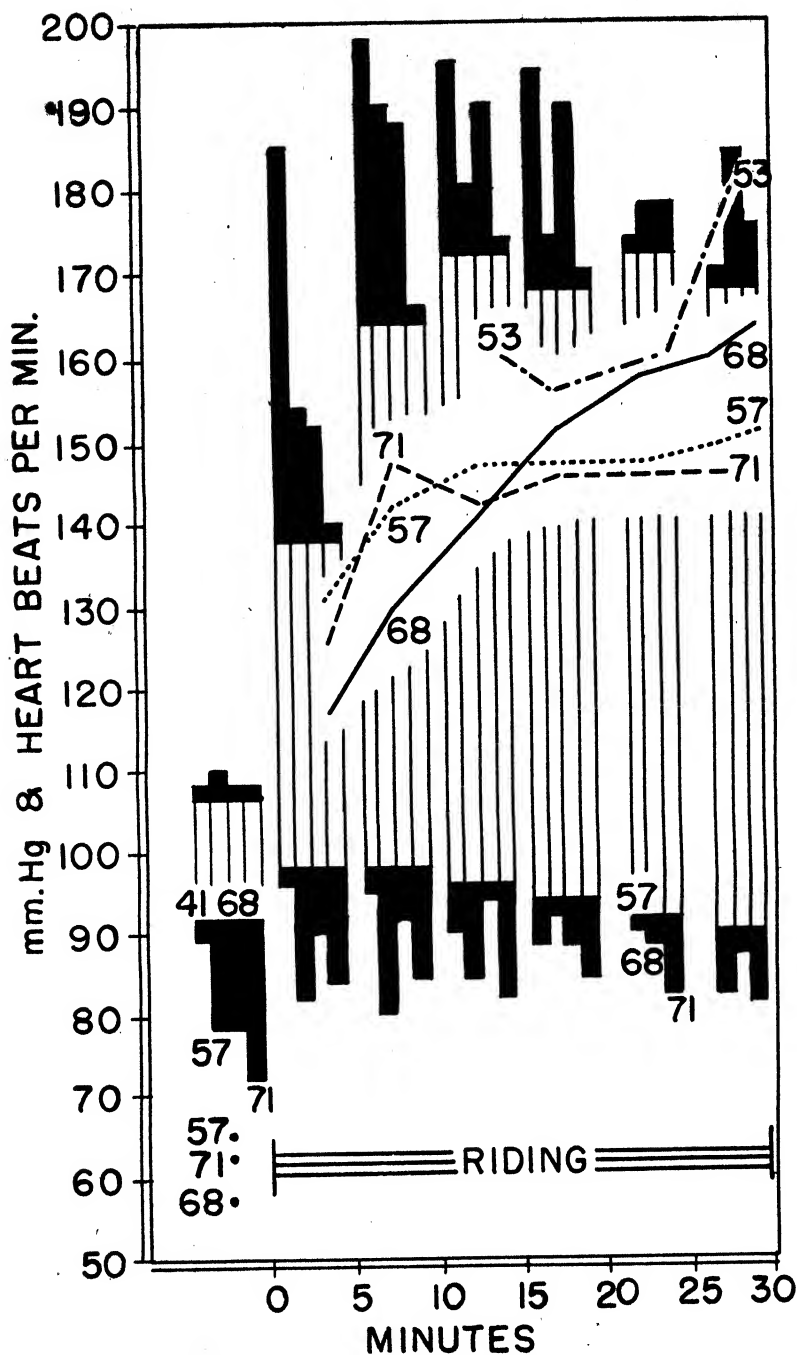


Fig. 1. Effect of age upon circulatory reaction while riding cycle-ergometer. The thick vertical lines (interrupted in the middle for convenience) represent pulse pressure, the upper end of each being at the systolic level, the lower at the diastolic level. Determinations were made before the ride and every five minutes during the ride which lasted 18 minutes for 41 and 30 minutes for 57, 68 and 71. The dots represent pulse rate determinations before riding. The four lines crossing the figure are the pulse rates corresponding to 53, 57, 68 and 71.

For further explanation see text.

Here systolic pressure is distinctly higher at 41 and so is the diastolic. But one does not see any difference in the trends throughout the experiment which might be attributed to increasing age.

The pulse product times one and a half ( $p.p. \times p.r. \times 3/2$ ), which one of us likes to persuade himself is an index of minute volume, was calculated for 57, 68 and 71—p.r. was not determined at 41 nor p.p. at 53. But the results were not illuminating and their discussion would not be profitable at the present time. Suffice it that the curves at 57 and 71 resembled each other while that at 68 differed considerably from the other two.

"Double rides." In two series of experiments designed to clarify the process of "warming up" several subjects were directed to ride both morning and afternoon so that the effect of the morning ride upon the afternoon ride could be observed. This work is not yet ready for publication but in so far as the results pertain to the problems of age they are as follows:

AGE	"DOUBLE RIDES" IN RELATION TO AGE		
	a.m. Rides	p.m. Rides same day	Improvement
	kg.m.	kg.m.	%
57	29,372	30,338	3.6
68	22,300	23,000	3.58

More experiments are needed before a discussion of those already performed would become profitable. Suffice it for the present that in this subject the picture was not altered between 57 and 68 years. Such rides were not performed at 71.

*Resting systolic pressure.* Other observers state that the resting systolic pressure remains essentially unaltered throughout life (6). Observations on the subject of these experiments accord with this. At 41 systolic pressure on rising in the morning, comfortably seated, was 106.5 (av. of 111 determinations,  $\pm 4.82$ , coef. of variability 4.51). At 68 at the end of prolonged recumbency systolic pressure was 109.02 (av. of 7 determinations,  $\pm 4.24$ , coef. of variability 3.88). Furthermore systolic pressure before exercise, sitting on the cycle-ergometer, was 126 at both 41 and 68.

*Pulse rate during training.* It is well known that the resting pulse rate is usually decreased as the result of training. The minimum resting pulse rate at 41 (3-mile run) was 47; at 42 (cycle-ergometer) 52 but subsequently (tennis) 47; at 71 (cycle-ergometer) 52.

*Recovery time and margin of safety.* The criterion of recovery is the ability to do the same task over again. It is when there is no "hang over." At 57 the subject not infrequently rode on two consecutive days. At 68 an interval of two rest days (e.g., rides on first and fourth) was tried but the subject was dissatisfied and increased the rest period to three such days. At 71 the interval was never less than two rest days, generally more than four. The judgment of

recovery was of course made on subjective data only and the desire was not to repeat the performance as soon as possible but to be sure that no "hang over" would viciate the next experiment. The difference between 57, on the one hand, and 68 and 71, on the other, was subjectively unmistakable.

The factor of safety which may be defined as the margin between the maximal ride and the ride which ends in collapse, actual or impending, is not easy to determine. There were on three occasions during the first decade of the century symptoms which brought the subject suddenly to recumbency. Having learned his lesson, he avoided trouble henceforth except on the occasion of October 29 at 68 years. But, if we grant that the preparation at 68 was inadequate, a narrow margin would not be surprising and any conclusions regarding the relation of age to margin would be unjustifiable.

The subject believes that his margin is narrower than it used to be. He feels not only that every form of effort "takes more out of him" than formerly, but also that only a little extra-exertion would "lay him out flat." If he is correct, then this margin of safety is decreased. But all this may be pure fancy. It is possible that a study of the negative phase (the circulatory depression during recovery) might be illuminating, but such a study has hardly been begun.

#### SUMMARY AND CONCLUSIONS

Observations on the capacity for physical work and the cardio-vascular reactions during exercise were made upon a single subject. The latter rode a cycle-ergometer at 41, 53, 57, 68 and 71 years of age. During these rides the arterial blood pressures and pulse rate were usually determined and the external work done was calculated for each ride. The results obtained support the following conclusions.

1. With age working capacity fell off, becoming at 71 years about 50 per cent of what it had been at 41 years.

2. When two rides are performed on the same day the score in the afternoon is greater than the score in the morning by about 3.5 per cent at both 57 and 68 years.

3. The number of days necessary for complete recovery from a ride is greater at 68 and 71 than at 57.

4. The circulatory reaction during maximal performance is much the same at all these ages. At 41 the systolic pressure rose higher but the ride which produced this pressure was only  $\frac{2}{3}$  as long as the rides performed later and the tempo was much higher.

5. The resting values of the systolic pressure whether lying or sitting on the cycle-ergometer did not change between 41 and 68.

6. The maximum reduction of the resting pulse rate due to training is about the same at 41 and at 71 years.

7. In training for a test on the cycle-ergometer the best preparation is to ride frequently upon this apparatus or to perform 3-mile runs. Road walking and mountain climbing do not yield as good results.

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# SEASONAL CHANGES IN FOOD CONSUMPTION AND RATE OF GROWTH OF THE ALBINO RAT

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In the course of experiments in this laboratory concerning the influence of different diets upon the complete life history of the albino rat, many records of food intake have been collected. In the case of the diets which have had as their chief ingredients ground whole wheat and whole milk powder, these supplied in different proportions or with supplements of added minerals or vitamin materials, differences were found in the efficiency during the period of early growth, but the calorie intake for the adult was practically the same. This ranged from 18 to 21 calories per 100 grams of rat per day. Small, irregular variations occurred on all diets, which seemed to have no definite relation to the age of the rat. It was thought that these might be associated with changes in season, and an investigation of that question is reported here.

For this study records of food intake of one diet only were used, and as a larger number of records for our diet 13 (also known as diet B) were available, these were the ones selected.

In 1925 a study of five year records for adult rats, six months of age and over, showed a seemingly significant difference in food intake in winter and summer months. This study was carried no further at that time. In this report all of the data on diet 13 experiments carried on from 1919 to 1929, and 1932 to 1939 are included.

These data were first organized into month groupings. The number of cases in each group was over 300 and a regular decline was seen from January to July, values going from 21.4 calories per 100 grams of adult rat per day in January through 20.9, 20.7, 20.5, 20.3, 19.7 to a low of 19.1 for July, rising again through 19.3, 20.4, 21.4, 21.2, to 21.5 for December. Since no difference great enough to be significant was apparent between any two successive months, these data are not given, but it is very evident that there was a distinct trend downward between mid-winter and mid-summer, and *vice versa*.

If, instead of using a month interval, a 3-month interval is used as in table 1A, this difference is clearly seen. The largest number of calories per 100 grams of rat is required during the winter months, December, January and February, the lowest during the summer months, June, July and August—this in spite of the fact that under our laboratory conditions there is very little difference between the room temperatures in the winter and in the summer. The intakes during the spring and fall are quite similar to each other and lie between those for winter and summer.

The number of cases in each of the four seasons is between 968 and 1066, large enough and similar enough to justify statistical interpretation. Reference

to the table shows the difference between the average number of calories per 100 grams of rat per day in the winter months and the summer months to be 1.9, with a probable error of 0.072. This difference, being over 26 times its probable error, is undeniably significant.

The frequency distributions for these two periods are given in figure 1. They show the smoothness of these distributions, and the shift of the mode in line with the change in the mean.

The differences between winter and spring, spring and summer, summer and fall with their probable errors are:  $0.8 \pm 0.075$ ,  $1.1 \pm 0.06$ ,  $1.6 \pm 0.07$ , respec-

TABLE 1  
*Calories per 100 grams of rat per day by season*

	DEC. TO FEB., INC.	MAR. TO MAY, INC.	JUNE TO AUG., INC.	SEPT. TO NOV., INC.
A. Adults				
No. cases.....	997	1066	987	968
Mean $\pm$ P.E.....	$21.3 \pm 0.06$	$20.5 \pm 0.05$	$19.4 \pm 0.04$	$21.0 \pm 0.05$
C.V.....	12.5	11.8	10.5	11.7
B. Young growing rats				
5th and 6th week				
No. cases.....	82	101	136	172
Mean $\pm$ P.E.....	$51.7 \pm 0.49$	$48.7 \pm 0.39$	$46.0 \pm 0.35$	$48.8 \pm 0.33$
C.V.....	13.0	12.0	13.4	13.4
7th and 8th week				
No. cases.....	82	102	135	174
Mean $\pm$ P.E.....	$42.0 \pm 0.31$	$41.2 \pm 0.30$	$39.6 \pm 0.30$	$42.1 \pm 0.26$
C.V.....	10.2	11.1	13.2	12.1
C				
5th to 8th week				
No. cases.....	82	101	134	173
Mean $\pm$ P.E.....	$46.8 \pm 0.37$	$45.0 \pm 0.31$	$42.7 \pm 0.29$	$45.4 \pm 0.26$
C.V.....	10.9	10.3	11.8	11.6

tively. These differences are all many times their probable errors, hence undoubtedly significant. This change in food intake as related to season is shown graphically in figure 2A.

The differences thus found for adults are so striking and show such a smooth distribution that it was decided to investigate the question of a possible seasonal effect upon food intake of the rapidly growing young rat. For reasons which will be apparent later, the 4-week period of most rapid growth, the 28th to 56th day of life, was selected. The food intake expressed as calories per 100 grams of rat is about double that of the adult rat during the first two of these weeks, and drops rather rapidly in the next two, for that reason the food intake is

usually stated in 2-week intervals rather than 4-week as for adults. It is given in both ways for 3-month periods in table 1, B and C.

It will be noticed that the same seasonal differences are observed for rapidly growing young as for adults, the food eaten per 100 grams of rat per day being significantly less in the 3 months June to August than for any other 3-month period, and highest in the 3 winter months. For the 5th and 6th week of life, this difference is 5.7, with a probable error of 0.6; for the 7th and 8th week, it is 2.4 with a probable error of 0.4. For the entire 4-week interval covering the

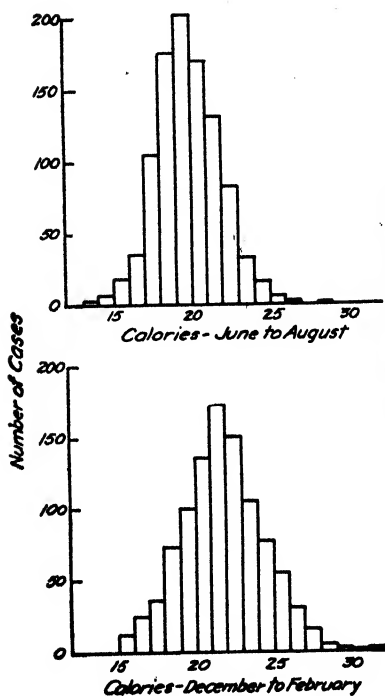


Fig. 1

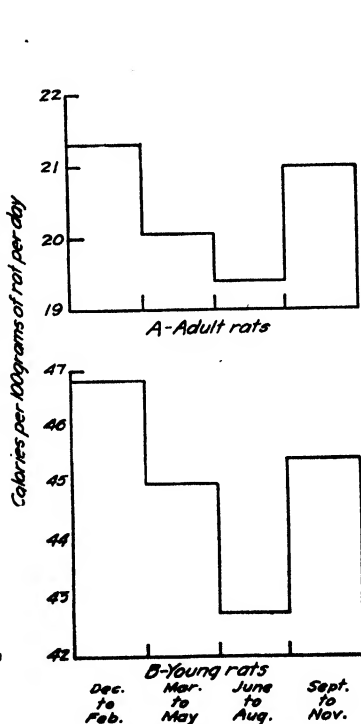


Fig. 2

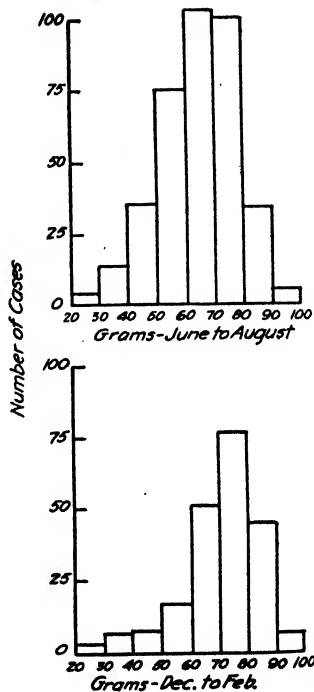


Fig. 3

Fig. 1. Calories per 100 grams of rat per day

Fig. 2. Intake by seasons

Fig. 3. Gain of female rats, 28th-56th day of life

5th to 8th week of life, the difference is 4.1 with a probable error of 0.47. These differences are all statistically significant. The smoothness with which this graph parallels that of the adults is seen in figure 2B.

In 1927 Dr. O. H. M. Gloy<sup>1</sup> showed the growth of young rats to vary with season. The finding of the seasonal difference in the food intake of both the adult and the young growing rat made it seem desirable to see how these were related to growth. As in the food calculations for the young rat summarized

<sup>1</sup>GLOY, O. H. M. Dissertation, Columbia University, 1927.

above, the time interval from the 28th to the 56th day is used in computing early growth.

Doctor Gloy's data with a large number of additional cases covering the same period as in the adult study were used. These were grouped by monthly intervals first, then by seasonal 3-month periods. As in food intake, no statistically significant differences occur between successive months, so to save space only the 3-month interval values are given in table 2. The greatest gain for both males

TABLE 2  
*Gain of young rats (28th to 56th day) as related to season*

	DEC. TO FEB., INC.	MAR. TO MAY, INC.	JUNE TO AUG., INC.	SEPT. TO NOV., INC.
Males				
No. cases.....	159	202	305	389
Mean $\pm$ P.E.....	92.4 $\pm$ 1.06	88.9 $\pm$ 0.86	82.3 $\pm$ 0.75	83.6 $\pm$ 0.66
C.V.....	21.8	20.7	23.9	23.2
Females				
No. cases.....	216	289	369	517
Mean $\pm$ P.E.....	70.4 $\pm$ 0.63	67.0 $\pm$ 0.50	64.1 $\pm$ 0.46	66.1 $\pm$ 0.35
C.V.....	19.6	18.9	20.9	18.1

TABLE 3  
*Efficiency of food utilization in different season*

	DEC. TO FEB., INC.	MAR. TO MAY, INC.	JUNE TO AUG., INC.	SEPT. TO NOV., INC.
A. Gain per 1000 calories				
No. cases.....	83	102	137	180
Mean $\pm$ P.E.....	80.2 $\pm$ 0.62	81.2 $\pm$ 0.55	81.5 $\pm$ 0.50	76.7 $\pm$ 0.48
C.V.....	10.6	10.2	10.8	12.5
B. Calories per gram gain				
No. cases.....	83	102	137	180
Mean $\pm$ P.E.....	12.6 $\pm$ 0.11	12.4 $\pm$ 0.09	12.4 $\pm$ 0.08	13.2 $\pm$ 0.08
C.V.....	11.9	10.7	11.2	12.8

and females was made in the three winter months, the lowest in the three summer months. For the males this difference is  $10.1 \pm 1.30$ , with a critical ratio of 7.8; for the females it is  $6.3 \pm 0.78$ , critical ratio of 8.1. Both of these differences are statistically highly significant. The shift in the modal group and the smoothness of the distribution is shown in the two frequency distributions for the females (fig. 3). Also, in the case of both males and females the gain in the spring is nearer that of the winter, while in the fall it rises only slightly above that for the summer. The close parallelism of data for the two sexes is shown



in figure 4. The drop from the winter to spring and spring to summer follows the drop in food consumption as shown in figure 2B, but the food intake in the fall rises to that of the spring figure, while the gain does not go up as rapidly.

Whether there is a more efficient utilization of the food at one season than another was determined by comparing gain per 1000 calories of food consumed, or its converse, the number of calories required to give 1 gram of gain, for the different seasons. No difference between month to month values appears statistically significant when standing alone, but there is ample evidence of a seasonal difference as given in table 3 and shown in figure 5. It will be noted that the most efficient use of the food is in the summer months when the smallest gain is made, and the least food eaten. The least efficient 3-month period follows this directly. The differences which are great enough to be statistically significant when standing alone are those between spring and fall, and summer

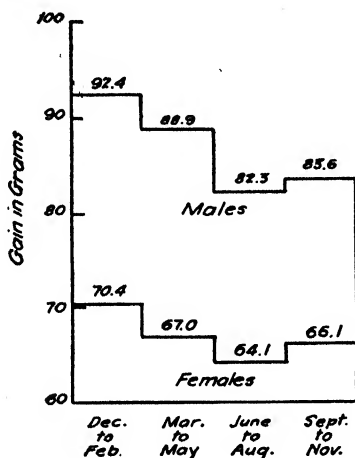


Fig. 4

Fig. 4. Gain of young rats—28th to 56th day—as related to season

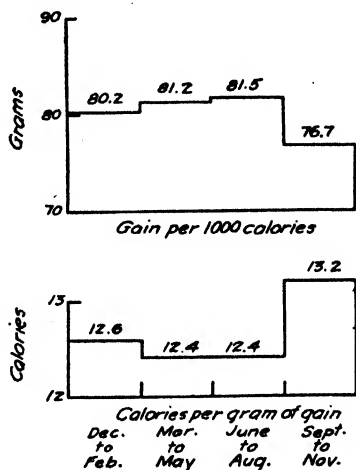


Fig. 5

Fig. 5. Efficiency of food utilization in different seasons

and fall. These differences do not correlate with differences in gain—whether this can be explained by a lag in the effect of food is uncertain. But in day to day observations over many years of study, it has been noticed that during a very hot period which immediately causes a lowered food intake, the loss of weight or slowing up of gain may not come until the following week, and *vice versa* when a sudden cold period causes an increase in food intake.

#### SUMMARY

A careful survey of food intake of a large number of rats maintained upon the same diet over a period of years shows the amount of food consumed to vary with the season of the years, the largest intake being in the winter months, the smallest in the summer months. The difference is unmistakably an actual difference, it being many times its probable error. This is established for the

adult rat, 6 months of age and over, by data including over 900 cases for each 3-month season, and for the rapidly growing rat, 28th to 56th day of age, by 82 to 173 cases.

It is also shown that the rate of growth of the young rat varies in the same way, the greatest gain being made in the winter months, the least in the summer. This is established for both males and females by groups of 159 to 517 cases each.

The food is used most efficiently in the summer as shown by gain made per 1000 calories, least efficiently in the fall.

These findings bring out more forcibly the importance of one rule that has always been followed in planning long time experiments in the Columbia laboratories—namely: that animals must be chosen to represent all seasons of the year so that such differences as those just reported will be ruled out.

## BLOOD REGENERATION IN PYRIDOXINE-DEFICIENT RATS

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Severe anemia due to pyridoxine deficiency has been produced in the dog (1) and pig (2). In pyridoxine-deficient rats, on the other hand, only a slight reduction in hemoglobin concentration has been found (3). This has caused some uncertainty regarding the importance of pyridoxine in erythropoiesis in the rat.

In the present studies we have succeeded in producing a moderately severe anemia in a small percentage of pyridoxine-deficient rats. In addition, an impairment in the rate of red blood cell regeneration has been demonstrated in most pyridoxine-deficient rats by subjecting these rats to the stress of repeated hemorrhage.

**EXPERIMENTAL.** Albino rats of Wistar and Osborne and Mendel strains were fed a pyridoxine-deficient diet no. 948<sup>1</sup> at weaning. In experiment 1 rats were observed for the spontaneous development of anemia. In experiments 2 and 3 rats were subjected to repeated hemorrhage in order to study the rate of red blood cell regeneration. The technic of Tabor, Kabat and Rosenthal (5) for bleeding small animals was used as before with minor changes (6). A volume of blood equivalent to 2 per cent of the body weight was removed from the tail of the rat each day for 3 consecutive days. Hematocrits were taken prior to each bleeding and at 2, 4, 6, 10 and 22 days after the last hemorrhage. The quantity of hemoglobin removed in bleeding was determined by an acid hematin method.

*Experiment 1.* A group of 36 rats, including rats which served as pyridoxine-deficient controls in experiments 2 and 3, were fed the pyridoxine-deficient diet and observed during a 4 month experimental period. Micro-determinations (Van Allen) of the hematocrit were made at irregular intervals during this period. Three rats which died before hematocrit determinations were made were not considered. Since the 33 remaining rats were drawn from 3 separate experiments, the number of cases of spontaneous anemia noted in this group does not necessarily indicate the true incidence.

Marked anemia (hematocrit value of 27 vol. per cent or less) was noted to

<sup>1</sup> Diet no. 948 consisted of anhydrous dextrose 68.76 grams, Crisco 8.0 grams, casein (Labco) 18.0 grams, ferric citrate (iron 18.09 per cent) 1.16 grams, copper sulfate (5H<sub>2</sub>O) 0.08 gram, and salt mixture no. 550 (4) 4.0 grams. Into this diet were incorporated 1 mgm. of thiamine hydrochloride, 2 mgm. of riboflavin, 4 mgm. of calcium pantothenate, 2 mgm. of niacin, 200 mgm. of choline chloride, 0.001 mgm. of biotin and 0.4 mgm. of 2-methyl-1,4-naphthoquinone. Twice weekly each rat received a supplement of 0.25 cc. of corn oil containing 2000 units of vitamin A and 200 units of vitamin D (Natola) and once weekly 3 mgm. of  $\alpha$ -tocopherol in 0.03 cc. of ethyl laurate.

develop in 6 of the 33 pyridoxine-deficient rats (table 1). Hematocrit values under 40 vol. per cent were observed in 5 of the other 27 rats. Two rats in which the hematocrit had progressively declined to values of 16 and 21 volumes per cent were treated with 100 $\gamma$  of pyridoxine for each of 4 days<sup>2</sup>. The hematocrit values 4 days after the start of treatment were 31 and 39 volumes per cent and at 10 days were 42 and 47 volumes per cent respectively. One rat with a hematocrit reading of 25 volumes per cent and similarly treated had a value of 22 volumes per cent 4 days after the start of treatment and died on the tenth day after the start of treatment. In the remaining rats with low hematocrit values which were untreated, no increase in the hematocrit values occurred until death.

Granulocytopenia (less than 500 polymorphonuclear cells per cu. mm.) was noted in 4 rats in this group. Total white blood cell counts were 350, 1100, 8850 and 9150 per cu. mm.; polymorphonuclear cell counts were 0, 50, 200 and 450 per cu. mm., respectively.

Dermatitis (mild to severe) was noted in most rats. Convulsions and paralysis were observed in only a few rats.

TABLE 1  
*Hematocrit values of pyridoxine-deficient rats*

LOWEST HEMATOCRIT VALUE	NO. OF RATS	NO. OF DAYS FROM START OF EXPERIMENT TO LOWEST HEMATOCRIT
vol. %		
16, 21, 23, 24, 25, 27*.....	6	62, 74, 33, 63, 55, 38
33, 35, 36, 36, 38*.....	5	69, 53, 72, 33, 28
40 to 54.....	22	49 to 120

\* These hematocrit values were noted after a progressive decline. Spontaneous increases in hematocrit were not noted. Figures for individual animals are given in corresponding order in columns for hematocrit and number of days.

*Experiment 2.* Male, weanling rats (8 groups of 3 litter mates) were distributed according to litter into 3 groups (A, B, C). The average weights of the 3 groups were equal. Rats in all 3 groups were fed the pyridoxine-deficient diet. Rats in group B received a daily oral supplement by pipette of 100 $\gamma$  of pyridoxine hydrochloride. After this regime had been maintained for 28 days in order to develop some degree of pyridoxine deficiency in the non-supplemented groups (A and C), rats in group A and B were subjected to hemorrhage as described. Litter mates in group C were not bled and served as pyridoxine-deficient controls. Food intake was "paired" in each litter.

Red blood cell regeneration was found to be much slower and less adequate in the rats (group A) fed the pyridoxine-deficient diet than in the pair-fed litter mates given the supplement of pyridoxine hydrochloride (group B) (table 2). Two rats in group A failed to regain normal hematocrit values even after a 22 day recovery period. Pair-fed control rats (group C) not bled and not supple-

<sup>2</sup> Food intake during the 10 day period after the start of treatment was limited to the intake of the 10 day period before treatment.

TABLE 2

*Inadequate erythropoiesis following hemorrhage in pyridoxine-deficient rats, compared with pair-fed, litter mates supplemented with pyridoxine*

RAT NO. §	HEMATOCRIT VALUES (VOL. %)							
	Hemorrhage period*			Recovery period				
	Values prior to each hemorrhage			Values at 2, 4, 6, 10 and 22 days after 3rd hemorrhage				
	1st hem.	2nd hem.	3rd hem.	2	4	6	10	22
Group A. † No pyridoxine supplement								
1A	50	33	23	25	34	37	46	50
2A	44	34	26	26	35	40	46	43
3A	48	31	19	17	28	Dead		
4A	45	31	20	18	24	30	40	48
5A	52	33	18	15	20	30	40	50
6A	44	31	20	16	21	22	22	30
7A	41	31	25	23	Dead			
8A	49	32	19	20	23	26	31	32
Average....	46.6	32.0	21.3	20.0	26.4	30.8	37.5	42.2
Group B. 100 γ pyridoxine daily								
1B	45	34	20	27	36	41	48	42
2B	51	38	28	29	37	44	47	45
3B	44	32	19	26	40	45	44	48
4B	43	28	23	26	35	41	46	48
5B	43	34	24	27	32	35	38	44
6B	53	34	23	24	37	43	48	54
7B	46	34	22	29	38	40	47	52
8B	44	35	26	31	40	43	45	45
Average....	46.1	33.6	23.1	27.4	36.7 ‡	41.2 ‡	45.3 ‡	46.3 ‡
S.E. diff. ¶				1.8	2.5	3.0	4.2	

\* The average total hemoglobin loss in group A was 0.37 gram and in group B 0.41 gram.

† Eight rats (group C) were treated exactly as litter mates in group A but were not bled. Average hematocrit values of rats in group C at 35 and 53 days after starting the experimental diet were 48.4 and 42.3 volumes percent respectively. Food intake was paired according to litters in all groups (A, B, C). Average weights of rats in groups A, B and C were 38.3, 39.6 and 39.5 grams respectively at the start of the experimental diet, 53.9, 55.3 and 53.8 at 28 days, and 55.2, 61.2 and 53.5 grams at 53 days after starting the experimental diet.

‡ Hematocrit values for rats, whose litter mates had died, were not included in this average.

§ Numerals indicate litter numbers; letters indicate groups.

¶ Standard error of difference between averages.

mented with pyridoxine hydrochloride all survived this period but did not develop anemia during this time.

*Experiment 3.* A group of 26 rats was fed the pyridoxine-deficient diet for

TABLE 3

*Inadequate erythropoiesis following hemorrhage in pyridoxine-deficient rats compared with pair-fed, litter mates supplemented with pyridoxine for only a limited period*

L. casei factor\* administered to both groups

RAT NO.	HEMATOCRIT VALUES (VOL. %)							
	Hemorrhage period†			Recovery period				
	Values prior to each hemorrhage			Values at 2, 4, 6, 10 and 22 days after 3rd hemorrhage				
	1st hem.	2nd hem.	3rd hem.	2	4	6	10	22
Group A.‡ No pyridoxine supplement								
10A	52	38	25	24	30	37	41	43
11A	45	42	27	31	38	45	49	48
12A	45	34	22	22	27	34	41	Dead
13A	43	30	18	17	27	33	42	Dead
14A	42	37	33	19	23	40	Dead	
15A	44	32	21	16	Dead			
16A	43	37	25	Dead				
17A	47	35	26	20	30	35	44	Dead
18A	43	32	24	24	33	39	43	48
Average....	44.9	35.1	24.5	21.9¶	29.7¶	38.0¶	43.2¶	46.3¶
Group B. 100 γ pyridoxine daily§								
10B	50	46	29	32	42	43	47	50
11B	52	40	27	30	41	43	48	50
12B	45	41	30	35	49	49	52	54
13B	41	40	28	31	38	43	42	48
14B	54		22	32	45	51	52	58
15B	42	37	28	31	33	36	49	52
16B	45	37	33	40	45	49	51	54
17B	46	40	31	Dead				
18B	47	39	25	32	42	47	51	52
Average....	46.9	40.0	28.1	31.9¶	42.8¶	46.0¶	48.0¶	50.7¶
S.E. diff. **				2.0	2.6	2.3	2.3	

\* Crystalline L. casei factor (10 gamma) was administered daily to all groups for 10 days starting 2 days before the first hemorrhage.

† The first hemorrhage was 36 days after starting the experimental diet. Average total hemoglobin loss in group A was 0.37 gram and in group B 0.43 gram.

‡ Four rats (group C) were treated exactly as litter mates in group A but were not bled. The average hematocrit values at 33 and 45 days after starting the diet were 51 and 49 volumes percent respectively.

§ Pyridoxine supplements were started 2 days before the first hemorrhage, and continued throughout the remainder of the experiment. All groups were pair-fed after starting the pyridoxine supplement.

¶ Hematocrit values for rats whose litter mates had died were not included in this average.

|| Numerals indicate litter numbers; letters indicate groups.

\*\* Standard error of difference between averages.

33 days. At this time, the rats were distributed according to litters into 3 groups (A, B and C containing, 9, 9 and 4 rats respectively) of approximately equal average hematocrit values. (Four rats with hematocrit values under 40 volumes per cent were excluded from this experiment, and have been included in experiment 1.) Rats in groups A and B were litter mates. Rats in group C were from 4 of the litters represented in groups A and B. Rats in group B received a daily oral supplement of 100 $\gamma$  of pyridoxine hydrochloride. All groups received a special daily oral supplement of 10 $\gamma$  of crystalline L. casei factor<sup>2</sup> ("folic acid") to make for a more complete vitamin supplement. After 2 days' administration of these supplements, rats in groups A and B were subjected to hemorrhage and rats in group C served as pyridoxine-deficient controls. The L. casei factor supplement was administered for only 10 days; the pyridoxine hydrochloride supplement (to rats in group B) was continued throughout the remainder of the experiment. Food intake was "paired" as in experiment 2.

The data of this experiment (table 3) indicate that the inadequate erythropoiesis in rats fed the pyridoxine-deficient diet was prevented by pyridoxine hydrochloride even when administration was begun only 2 days before the hemorrhage period.

Since both experiments 2 and 3 were conducted with litter mates identically treated except for pyridoxine supplements, the most important comparison is between a pyridoxine-deficient rat and its pair-fed, pyridoxine-supplemented litter mate. It is noteworthy, however, that in addition to these individual comparisons the average hematocrit readings of the supplemented group were significantly greater than those of the unsupplemented group during the period of red blood cell regeneration following hemorrhage (tables 2, 3).

**DISCUSSION.** The importance of pyridoxine in erythropoiesis in the rat has been suggested by the work of Fouts and Lepkovsky (3) and indicated in the present studies by the occasional occurrence of a moderately severe anemia in rats fed a pyridoxine-deficient diet. Additional evidence has been obtained by subjecting rats fed this pyridoxine-deficient diet to the stress of repeated bleedings. An impairment in the rate of red blood cell regeneration has been found uniformly in these rats when compared with litter-mate, pair-fed controls supplemented with pyridoxine. This erythropoietic inadequacy was prevented by pyridoxine even when administration was begun only 2 days before the start of hemorrhage.

These findings for pyridoxine are similar to, though less striking than, those reported for L. casei factor ("folic acid") (6). In those studies the bleeding technic was likewise used to manifest an erythropoietic failure which usually remained latent.

#### SUMMARY

1. Moderately severe anemia has been found to occur occasionally in pyridoxine-deficient rats.

<sup>2</sup>The crystalline material was furnished through the courtesy of E. L. R. Stokstad, B. L. Hutchings and N. H. Slobodkin of Lederle Laboratories.

2. A latent erythropoietic inadequacy indicated by an impairment in the rate of red blood cell regeneration after hemorrhage has been demonstrated in pyridoxine-deficient rats.

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# THE TRANSMISSION BY CROSSED CIRCULATION OF A SHOCK PRODUCING FACTOR<sup>1</sup>

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The evidence for the toxemic theory of shock elaborated by Cannon and Bayliss (1, 2) and the subsequent controversies over this theory need not be reviewed here. In later attempts to find convincing evidence that a toxemic factor exists, blood and tissue extracts from both normal animals and from those that had been subjected to shock producing manoeuvres have been injected into other animals. In addition, attempts have been made to induce shock by crossed circulation and the perfusion of animals with blood from the traumatized tissue of another animal. McIver and Haggard (3) established a crossed circulation between a cat and the previously traumatized legs of another cat. In 9 out of 12 such experiments the blood pressure fell to a shock level (a persistent mean pressure of below 70 mm. Hg), whereas in 4 out of 5 controls, using untraumatized legs, it did not. The experiments lasted from  $\frac{1}{2}$  to  $1\frac{1}{2}$  hours and there were no deaths. Vaccarezza (4) having produced a thermal injury to the leg of one dog (A) made a vascular union with another dog (B) so that the blood from the injured leg perfused dog B but not the rest of dog A. Clotting in the system terminated the crossed circulation within "a few hours." In the 2 experiments performed, dog B died in 5 and 8 hours respectively, at which time dog A was still alive. The control experiment of perfusing through a normal leg was not performed, nor was the possibility of death from embolism ruled out. Fender and Guptill (5), criticizing these findings, repeated Vaccarezza's experiments with negative results. Kendrick, Essex and Helmholtz (6) perfused dogs with oxygenated blood from the normal and traumatized legs of other dogs. In two control experiments, using blood from normal legs, no appreciable change in blood pressure or hematocrit occurred during the experimental period of 3 or 4 hours, but the recipient animals died at 8 and 18 hours respectively after the experiment. In four experiments where traumatized limbs were used, two animals died during the experimental period, while a third dog died 8 hours and a fourth 15 hours after the experimental period. The authors stressed the lack of severe reactions in the control animals during the period of observation, and concluded that their experiments "furnish suggestive, but not conclusive evidence for the toxemia theory of shock." Best and Solandt (7) in a report on therapy in shock, state "we have shown that the blood of a shocked dog will frequently, when passed in large quantities into a normal dog through a special pressure separating exchange transfusion pump, produce a fall in the blood pressure of the normal dog. In every such case the exchange of the bloods of the two animals, prior to the onset of shock, had no effect on the

<sup>1</sup> Aided by a grant from the Charlton Research Fund.

blood pressure of either." On the basis of these and other considerations, Best and Solandt favored the view that a circulating toxic factor exists. We have recently (8) reported on the production of tourniquet shock in the rabbit with a fairly predictable survival time. The present communication deals with the transmission of this form of shock to another animal.

**METHODS.** Rabbits weighing 2 to 3 kilos were used. Anesthesia was induced by 15 mgm. of nembutal intravenously, and was lightly maintained by the concomitant administration of 1.5 grams of urethane per kilo subcutaneously. Rarely additional small amounts of nembutal were required, but deep anesthesia was avoided. Heparin (Abbott)<sup>2</sup> was added from time to time to prevent clotting, about 3 mgm. per kilo per hour being given. This precaution is particularly advisable in the rabbit. Its administration has no effect on the development of tourniquet shock. We established a vascular union between the general circulation of one rabbit (the recipient) and the hind legs of another (the donor), a certain amount of lumbar and sacral tissue being unavoidably included in the circulation of the latter. In the test experiments the legs of the donor were subjected to tourniquet occlusion of  $2.1 \pm 0.2$  hours' duration; in the control experiments they were not. On the other hand, a certain delay between shutting off the circulation to the legs of the donor and establishing a crossed circulation was inevitable. We were able to reduce this anoxia to 28 minutes, and to keep it constant to within 5 minutes. The technique of establishing the crossed circulation was a modification of that of McIver and Haggard and a standard procedure was evolved with three main objects: 1, to keep the above mentioned period of anoxia in the legs of the donor small and constant; 2, to avoid hemorrhage; 3, to avoid clotting. The procedure follows: 1, both animals anesthetized; 2, *donor*: 3 mgm. per kgm. heparin; 3, *donor*: tourniquet applied to both legs (in "shock" experiments); 4, *recipient*: right femoral artery, left common carotid artery and left external jugular vein dissected out; 5, *donor*: (a) abdominal aorta and vena cava dissected out for a distance of 2 to 3 cm. about halfway between renal vessels and bifurcation of iliacs; (b) 3 mgm. per kgm. heparin; (c) right carotid cannulated for bleeding. 6, *recipient*: (a) right femoral artery cannulated for blood pressure; (b) left femoral vessels tied to exclude from circulation; 7, *donor*: (a) abdominal wall including skin clamped with overlapping Kelly clamps to prevent oozing, and cut diagonally from symphysis pubis to lumbar muscles below ribs; (b) mesentery and gut tied and cut away; (c) aorta and vena cava cannulated; (d) window cord tied tightly around lumbar muscles below kidneys; (e) animal bled to death into flask from carotid (see text); (f) tourniquets released (in "shock" experiments); (g) upper part of body cut away, the spinal canal being plugged with gauze; 8, crossed circulation connections, consisting of 2 lengths of z-shaped, 3 mm. bore glass tubing about 25 cm. long, plus rubber tubing, attached to appropriate cannulae without releasing flow; 9, *donor*: legs "filled" with heparinized blood (see text). 10, crossed circulation established.

The blood pressure of the recipient animal was continuously recorded, and

<sup>2</sup> We are indebted to the Abbott Laboratories for a generous supply of heparin.

hematocrit observations were made from time to time. The circulation time between the animals was noted at intervals by observing the time taken for an introduced small bubble of oxygen to traverse the glass tubing (of known volume) between the femoral vein of the donor and the jugular of the recipient. This procedure, incidentally, also served to verify the fact that the crossed circulation was still functioning.

**RESULTS.** These are summarized in table 1. The experiments are divided into two groups. In the first of these, the crossed circulation was established without making any attempt to counteract a possible loss of blood from the recipient into the legs of the donor. With the severance of the donor's spinal cord, a large increase in the circulatory capacity of the legs was to be anticipated. That the recipient did actually "bleed" into the legs of the donor was indicated by an immediate and almost invariable sharp fall in the blood pressure when the crossed circulation was established. This fall averaged 34 mm. Hg in the control experiments and 28 mm. Hg in the experiments in which a tourniquet had been previously applied to the legs. Usually the blood pressure tended to

TABLE 1  
*Survival times in tourniquet shock transmitted by crossed-circulation*

	CONTROL—NO TOURNIQUET ON LEGS OF "DONOR"	TEST—TOURNIQUET FOR 2 HRS. ON LEGS OF "DONOR"
1st group (No blood added to legs of "donor")	6.7 $\pm$ 1.6 hrs.* (8 expts.)	3.1 $\pm$ 0.4 hrs.* (6 expts.)
2nd group (Blood added to legs of "donor")	15.5 $\pm$ 2.3 hrs.* (7 expts.)	4.3 $\pm$ 0.4 hrs.* (7 expts.)

\* After establishment of the crossed-circulation.

$\pm$  Indicates standard mean deviation.

rise again, though not ordinarily to the previous level. Under these conditions, the average survival time of animals that received blood from legs that had previously been occluded was  $3.1 \pm 0.4$  hours. The survival time of the animals who were cross-circulated with blood passing through non-occluded legs was  $6.7 \pm 1.6$  hours. While these differences in time are possibly of statistical significance, they were not very satisfactory as proof of the transmission of a shock-producing factor.

Since the anesthetized animal with a mock operation will live, on the average, more than 24 hours, we felt the comparatively short survival in the control experiments to be due chiefly to two factors: the anoxia of the donor's legs for about  $\frac{1}{2}$  hour, and the "bleeding" into these legs from the recipient animal. In a second group of experiments, therefore, we bled the donor to death from the carotid, as indicated above, and introduced this heparinized blood, amounting to 30 to 35 cc., into the legs of the donor, at a pressure corresponding to the mean blood pressure of the recipient, before establishing the crossed circulation. It should be emphasized that all of this blood flowed in freely and the leg vessels un-

doubtedly would have held more. Nevertheless, with this change in technique, the immediate fall of blood pressure on establishing the crossed circulation was almost abolished (to an average fall of 6 mm. Hg). The survival time of the animals receiving blood from occluded legs was increased from 3.1 to  $4.3 \pm 0.4$  hours. This change was not marked, and indicated that though the "hemorrhage" had played a part, the dominant factor was still the occlusion of the donor's legs. This survival time is practically the same as the 3.8 hours which we have previously shown (*loc. cit.*) to be the average survival time of animals whose own legs had been occluded for a 2 hour period. In the control experiments of this second group, the animal receiving blood from non-occluded legs survived an average of  $15.6 \pm 2.3$  hours in 7 experiments. That this group did not survive as long as simply anesthetized and mock-operated animals is perhaps largely due to the unavoidable  $\frac{1}{2}$  hour anoxia of the donor's legs.

We have previously observed that in rabbits dying of shock following tourniquet occlusion, the hematocrit changes were negligible. The same is true in the recipient animals during the present experiments, the changes averaging  $-0.7 \pm 0.9$  in the "shock" animals and  $-3.6 \pm 0.3$  in the controls. Hence loss of fluid from the circulation could not have been the cause of death in these experiments.

The blood pressure records showed nothing of special interest except that the fall before death tended to become precipitous when the pressure reached a level of 30 to 40 mm. Hg. The flow through the crossed circulation quickly stabilized itself at a rate, very roughly estimated, of 4 to 5 cc. per 100 grams of leg tissue per minute. As time went on there was a gradual tendency to fall. There was no particular difference in the flow as between the control and the test experiments save as to duration, marked slowing of the circulation beginning to occur, as a rule, about an hour before death.

#### SUMMARY

1. The hind legs of rabbits were subjected to tourniquet circulatory occlusion, and then a crossed circulation was established between them and the general circulation of recipient rabbits.

2. The survival time of the latter was similar to that of animals whose own legs had been occluded for the same length of time. Hematocrit changes were negligible.

3. The evidence obtained indicates that a shock-producing factor was transmitted in the blood.

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# LIVER RESERVES OF VITAMIN A AND THEIR RELATION TO THE SIGNS OF VITAMIN A DEFICIENCY IN THE ALBINO RAT<sup>1</sup>

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The several physiological signs of vitamin A deficiency in mammals are too well recognized to require further description. Failure of growth and paralysis of the hind limbs in the young and xerophthalmia in both young and adult animals occur only after a prolonged period of complete deprivation of the vitamin, while night blindness and continuously cornified cells in the vagina of the female are less obvious signs and occur earlier in the course of the deficiency.

Both the occurrence and accurate measurement of night blindness in humans have been subjected to extensive study and the results have been used in the evaluation of their vitamin A status and requirements. It is assumed in these studies that an elevated threshold for dim light is evidence of the exhaustion of the vitamin A reserves of the liver; the length of time on the deficient diet required to produce this hemeralopic condition is regarded as a rough measure of the previous vitamin A reserves of the subject. It is obviously impossible to test these assumptions experimentally in human beings, though it would be of great interest to be certain of these facts. For this reason albino rats were used to determine the amount of the hepatic stores of vitamin A in animals showing symptoms of night blindness and of xerophthalmia. In addition, growth rates, oestrus cycles in the females, scotopic visual thresholds and vitamin A content of the livers were studied in rats receiving cod liver oil at several different levels of vitamin A intake.

**EXPERIMENTAL.** *General plan of the experiment.* Albino rats, weaned at 21 to 25 days, when weighing 30 to 45 grams, were placed on a vitamin A-free diet composed of 18 per cent extracted casein, 53 per cent cornstarch, 15 per cent brewer's yeast, 4 per cent Osborne-Mendel salt-mixture, 10 per cent cottonseed oil and irradiated ergosterol to supply three units of vitamin D value to each gram of diet. The experimental animals were offspring of mothers whose diet was somewhat limited in vitamin A content, thus insuring small vitamin A reserves in the young. From 3 to 6 animals were housed in a single large cage with raised screen bottom; the temperature of the room was relatively constant, varying from 76° to 80°F. Food and water were supplied ad libitum. The animals were divided into 4 groups of at least 20 rats each, males and females being equally represented. Litter-mates were distributed evenly throughout the groups. Each group received cod liver oil at a different level of vitamin A

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intake. Since the vitamin A requirement of animals shows a more nearly constant relation to weight than to any other bodily characteristic, the rats were weighed and the amount of vitamin A to be fed calculated on the basis of this weight once a week. Goss and Guilbert (1) have estimated that 18 to 22 units of vitamin A per kilogram of body weight per day will satisfy the rat's minimal needs for this dietary essential. This average minimal amount of 20 units per kilogram per day of vitamin A was given to one group and 30, 50, and 80 units, respectively, to the other three. The rats continued on this regimen until they were adult. As soon as the vagina opened, examinations were made at intervals on each female to determine whether the oestrus cycles were recurring normally. Several animals from each group were conditioned to respond to dim light and their visual thresholds measured. At approximately 275 days, when active growth had definitely ceased, some of the rats were killed and the amount of vitamin A in the livers measured. At this time the liver stores, even in animals on the higher levels of intake, were so meager that the remaining rats were kept to approximately 365 days of age, and at this time several of the animals that had been trained to respond to light were deprived of the vitamin A supplement and tested for hemeralopia at frequent intervals.

When the visual thresholds were abnormal beyond a doubt, some of these rats were killed and the livers analyzed. The remaining hemeralopic rats were continued on the vitamin A-free diet until xerophthalmia appeared before analyzing the livers. The untrained rats which remained were killed for liver analysis. Later, a second series of experiments was begun using three groups of animals which were given 10, 15, and 20 units of vitamin A per kilogram of body weight per day, respectively. The above procedure was followed except that no animals were depleted.

*Preparation and feeding of supplement.* U.S.P. reference cod liver oil no. 1 diluted with cottonseed oil containing 0.01 per cent hydroquinone, was used to supply the vitamin A supplements. Calculations of the amount of the reference oil to be fed were based on the assigned potency of 3000 international units per gram. Fresh dilutions of the stock oil were prepared every ten days and were stored at 0°C. between feedings.

*Vaginal examinations.* At intervals, beginning at the time of vaginal opening, the vaginal secretions of the female rats in this series were examined microscopically without staining.

*Visual threshold measurements.* It has been demonstrated by many investigators that responses based on brightness differentiation are easily acquired by the albino rat and that this particular kind of discrimination is fundamental for this species of animal. An apparatus very similar to that described by Slater and Munn (2) was built and used to determine the absolute threshold of the rat's dark-adapted eye.

The apparatus consisted, briefly, of a discrimination chamber whose floor sloped gently toward two stimulus patches separated by a narrow partition. The stimulus patches consisted of six-inch squares of flashed opal glass each of which could be illuminated separately from below. In front of the stimulus

patches was an electric grid through which a weak current, just sufficient to give a moderately unpleasant sensation to the feet of the rat, could be passed. Each of the stimulus patches formed the top of a light-tight box to the other end of which was fitted a slide carrying a seven watt Mazda lamp with an arrangement for placing filters and a diaphragm in front of it. The light was reflected upward through the glass plates by means of mirrors set at a  $45^\circ$  angle below them. The upper part of the apparatus was painted a dull black, while the inside of the light boxes was given several coats of white sphere paint which made an excellent surface for reflecting the light evenly over the entire plate. Tests made with the Macbeth illuminometer showed that the differences in brightness between the center and edges of the plate were negligible. The intensity of the light was varied by means of a series of decimal filters in combination with a calibrated diaphragm. The actual experimental lights used were of too small intensity to be measured directly. Instead, the lamp was calibrated without filters and with the diaphragm opened fully; the intensity of the experimental light was then calculated from the brightness of the lamp, the density of the filter, and the size of the aperture of the diaphragm.

The rat was trained to respond positively to light in the following way. He was presented with one stimulus patch light and the other dark. When he chose to go to the light side, there was a reward of food at the other end of the passage, but when the dark side was chosen, punishment followed in the form of a shock from the electric grid and food was withheld. After 100 to 200 trials, the rat would invariably choose the light and when he was responding with an accuracy of 100 per cent he was considered to be trained.

During the course of the tests the light was shifted from one side of the apparatus to the other in random order. The light patch was then reduced in intensity gradually until the point was reached at which the animal was responding with an accuracy of 80 per cent, but below which he was making many more errors. Each threshold reported here represents the average of 30 to 50 trials at the particular brightness on several days. Care was taken at all times to guard against all auditory or other cues which the animal might use in judging the correct response. If one persisted in presenting a light which was subliminal, the rat would finally refuse to run altogether or would indulge in aimless jumping. Morgan (3) has shown that the time required for complete dark adaptation is much longer for rats than for humans. For this reason, the rat was dark adapted for an hour before each test.

*Liver analysis.* The vitamin A content of the unsaponifiable fraction of the livers of the experimental animals was determined by means of the antimony trichloride reaction, using the Evelyn photoelectric colorimeter. The galvanometer readings were converted to vitamin A units by the use of a reference curve, derived from 20 analyses of the unsaponifiable fraction of reference cod liver oil no. 1. Complete absorption curves of a number of these unsaponifiable fractions were made by means of a Hilger constant wave length photoelectric spectrophotometer for comparison with the blue unit values.

*RESULTS. Growth and physiological condition of the animals.* It appeared

possible that a deficiency of dietary vitamin A might influence the weight gain of the rat at one particular period of growth more than at another. Accordingly for purposes of statistical analysis, weight gains made by the animals in each experimental group during 52 weeks of life have been divided according to five periods of time during each of which the rate of gain was relatively uniform, as shown by the characteristic slope of the growth curve at each period. The times studied represent weaning to 9 weeks, 9 to 13 weeks, 13 to 26 weeks, 26 to 36 weeks, and 36 to 52 weeks of age, respectively. A summary of the averages of these weight gains as well as of the occurrence of oestrus cycles in the females

TABLE 1

*The relation of vitamin A intake to growth response, oestrus cycles and visual threshold in rats*

VITAMIN A INTAKE, FED AS COD LIVER OIL	AVERAGE WEIGHT GAINS FOR SUCCESSIVE STAGES OF GROWTH, IN GRAMS PER WEEK, WITH STANDARD ERROR										OESTRUS CYCLES	VISUAL THRES- HOLDS* LOG $\mu$ LAMBERTS		
	Period I Weaning-9 wks.		Period II 9-13 wks.		Period III 13-26 wks.		Period IV 26-36 wks.		Period V 36-52 wks.					
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀		♀	♂	♀
<i>units/ kg./day</i>														
10	17.0± 1.27	15.0± 0.67	11.2± 1.24	8.3± 0.64	6.0± 0.78	2.6± 0.12						100% con- tinuous cornified cells	4.5 4.9	4.2 4.8
15	19.8± 0.64	15.9± 1.25	13.5± 0.64	9.1± 0.64	6.4± 0.49	3.7± 0.13						Some corni- fied cells in all smears	3.5 4.0	3.9 3.2 3.5
20	21.3± 0.98	15.8± 0.42	15.0± 0.70	8.7± 0.39	6.3± 0.43	3.2± 0.23	4.2± 0.45	2.4± 0.33	1.5± 0.21	0.90± 0.44	Normal	2.6 3.2 3.5	3.4 3.2 3.2	
30	21.5± 0.86	15.6± 0.56	16.3± 0.72	9.3± 0.44	6.9± 0.29	3.4± 0.12	3.6± 0.38	2.0± 0.76	1.7± 0.90	0.90± 0.10	Normal	2.6 3.5 3.7	2.5 3.2 3.2	
50	22.2± 0.93	17.6± 0.81	18.3± 1.11	9.9± 0.30	7.2± 0.30	3.5± 0.19	4.5± 0.40	2.6± 0.60	2.7± 0.22	1.6± 0.40	Normal	3.5 3.6 2.5	3.3 3.6 3.5	
80	23.6± 1.21	18.1± 0.36	18.8± 1.24	9.4± 0.38	6.5± 0.39	3.2± 0.20	4.5± 0.48	2.0± 0.25	2.0± 0.61	1.3± 0.42	Normal	2.5 3.5 3.6	3.4 2.6 3.2	

\* Values given are for individual rats, since the number of individual animals it was feasible to train and test was necessarily limited.

and visual thresholds of the individual rats on which such determinations were made, is given in table 1.

Individual t-tests were applied at each level of vitamin A intake for each period of growth. From this analysis, the effect of increasing amounts of dietary vitamin A appeared to be limited entirely to the periods from weaning to 9 weeks and from 9 to 13 weeks of age, that is, the time of active growth.

During the 9 weeks immediately following weaning, growth was significantly retarded in both male and female animals by levels of 10 and 15 units per kilogram body weight, as compared with the higher levels of vitamin A intake. In



this period of most rapid growth, the male animals appeared to be less sensitive to increasing amounts of vitamin A above the 20-unit level than did the females. There was a significant increase in rate of weight gain only between the 20- and 80-unit levels in the males, while the females showed significant increases between both 30 to 50 and 30 to 80 units of vitamin A per kilogram body weight. This difference in sensitivity between the sexes was reversed during the following period, when growth was somewhat slower. At this time the male animals showed significant differences between all levels except the 50- to 80-unit intervals while no significant differences between the several levels of intake were exhibited by the females.

Examination of the vaginal smears of female animals receiving vitamin A at the 10-unit level showed large numbers of cornified cells at all times. In fact, it was impossible to follow the oestrus cycles without staining. Those receiving 15 units exhibited some cornified cells in all smears, though not in sufficient numbers to obscure the normal cycles. Animals on the higher intakes were completely normal as far as vaginal contents were concerned, thus confirming the work of Goss and Guilbert (1).

Under the conditions of this experiment, the normal scotopic visual threshold of the rat appeared to be slightly higher than that reported for human beings. It is possible that the experimental procedure necessary to the determination in the rats has a tendency to give results which are somewhat high. Visual thresholds of animals on the two lowest levels of intake were elevated in relation to those on the higher levels.

In connection with the question of general physical well-being, two further observations are of interest. The rats on the two lowest levels of vitamin A intake had rough hair and the teeth were chalky white, with no sign of the usual orange pigmentation. Wolbach and Howe (4) have described the same condition in their vitamin A-deficient rats and ascribe it to a loss of the enamel layer and a change in the composition of the dentine.

In addition, the incidence of middle ear disease, which we have observed to be somewhat higher in the stock animals receiving limited amounts of vitamin A for the purpose of breeding young for bioassay, than in the stock colony as a whole, decreased with the increase of vitamin A intake and was greater in the males than in the females in almost every group. With the intake of 10 units per kilogram per day, the proportion of the group succumbing to middle ear disease was 44 and 38 per cent; with 15 units, 25 and 40 per cent; with 20 units, 40 and 18 per cent; with 30 units, 21 and 8 per cent; with 50 units, 13 and 0 per cent; and with 80 units, 8 and 10 per cent for the males and females, respectively.

*Vitamin A stores in the liver.* In table 2 is presented a summary of vitamin A stores of the animals on the four higher levels of vitamin A intake. The amounts of vitamin A stored were extremely meager in the rats receiving the vitamin at the 20-, 30-, and 50-unit levels. It is well-known that the Carr-Price reaction is not specific for vitamin A, and it was found in these experiments that all of the complete absorption curves of the liver extracts with the exception of the ones derived from livers of rats in the 80-unit group, gave marked indication of the

presence of substances other than vitamin A absorbing strongly in the region of 328 m $\mu$ . For this reason little quantitative significance can be attached to the actual figures except at the 80-unit level, though such differences as occur are in the expected direction. At the 80-unit level, measurable reserves are accumulated, although even they appear small in comparison with the tens of thousands of units which the rat liver is capable of storing. Goss and Guilbert (1) have reported similar results from experiments extending over a shorter period of time and in which they used animals which were first depleted of their stores to the point of the appearance of cornified vaginal cells. Baumann, Riising, and Steenbock (5) have shown that a smaller percentage of a large single dose of vitamin A can be recovered from the livers of animals that have been depleted than from normal animals. It was for this reason that these experiments were begun with weanling rats having small reserves, rather than with depleted and

TABLE 2

*The relation of vitamin A intake to vitamin A content of the livers of rats*

VITAMIN A INTAKE FEED AS COD LIVER OIL	AVERAGE VITAMIN A CONTENT OF LIVER WITH STANDARD ERROR											
	Animals 275 days old						Animals 365 days old					
	No. of animals		Units/whole liver		Units/gram liver		No. of animals		Units/whole liver		Units/gram liver	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
	<i>units/ kg./day</i>											
20	3	2	3.6	4.8	0.36	0.76	4	6	6.5	8.5	0.76	1.2
			±0.19	±0.40	±0.016	±0.059			±0.28	±0.11	±0.042	±0.25
30	6	5	4.3	4.9	0.46	0.75	5	7	5.9	7.0	0.61	1.01
			±0.47	±0.67	±0.060	±0.100			±0.87	±0.67	±0.070	±0.078
50	7	4	8.4	5.7	0.81	0.87	5	6	14.2	17.4	1.19	2.51
			±1.46	±1.02	±0.12	±0.092			±2.78	±4.37	±0.15	±0.82
80	5	3	45.5	62.4	4.65	12.2	5	6	98.4	104.6	9.0	15.9
			±42.6	±30.0	±2.64	±6.20			±34.2	±41.6	±4.10	±6.12

therefore physiologically abnormal animals. Analyses of the combined livers of eight male weanling rats and of three females gave an average value of 22.3 units per gram or 22.0 units per liver for the males and 23.8 units per gram or 24.4 units per liver for the females. Thus, the amount of the vitamin A in the livers of the rats on the three lower levels of vitamin A actually decreased from the time they were weaned until they were killed.

From these results it appears that until four times the so-called "minimum" dose of vitamin A is present in the diet of the rat, no measurable reserves are accumulated in the liver. It also may be noted that in spite of their smaller body size and hence lower vitamin intake, and the smaller size of the liver, the amount of vitamin A stored is greater in the females than in the males, not only on a per gram but also on an absolute basis.

*Depletion of vitamin A stores.* In table 3 may be found a summary of the observations made on the selected animals transferred to the unsupplemented

vitamin A-free diet after 365 days on one of the four highest levels of vitamin A intake. Continuous cornification appeared in the vaginal smears of the females so depleted immediately after the occurrence of hemeralopia, and was followed considerably later by the appearance of xerophthalmia. It is of interest that, although liver stores of vitamin A are considerably larger in the female than in the male, the female appears to deplete more quickly than does the male. Since the number of animals used in this portion of the experiment is small, the evidence is not conclusive; however, this result is not incompatible with the observation that the female is more sensitive to a relative deficiency of dietary vitamin A at an earlier period than is the male. The vitamin A content of the

TABLE 3

*The relation of previous vitamin A intake and liver stores in depleted rats to the appearance of hemeralopia and xerophthalmia*

EXPERIMENTAL ANIMAL AND VITAMIN A INTAKE	VISUAL THRESHOLDS		DEPLETION TIME REQUIRED TO PRODUCE:		VITAMIN A CONTENT OF LIVERS AT APPEARANCE OF:	
	Normal	Hemeralopic	Hemeralopia	Xerophthalmia	Hemeralopia	Xerophthalmia
	<i>log <math>\mu</math> lamberts</i>	<i>log <math>\mu</math> lamberts</i>	<i>days</i>	<i>days</i>	<i>units/whole liver</i>	<i>units/whole liver</i>
20 units/kg./day						
6526 ♂	3.5	4.5	9	23		5.2
6772 ♂	2.6	4.3	10	34		5.8
7037 ♀	3.2	4.2	5	25		13.3
30 units/kg./day						
6535 ♀	2.6	3.9	10	50		7.8
6771 ♂	3.5	4.5	38		13.3	
50 units/kg./day						
6773 ♀	3.3	4.1	48		6.0	
6770 ♂	2.5	3.9	48		7.6	
80 units/kg./day						
6774 ♀	3.4	4.5	55		7.2	
6769 ♂	3.6	4.7	83	112		5.0

livers of animals in a condition of mild vitamin A deficiency differed little from that of animals with xerophthalmia and neither was different from values for liver stores found in apparently normal animals receiving up to 50 units of vitamin A per kilogram per day. The fact that the 30-unit and 50-unit levels of intake prolonged considerably the time necessary to produce physiological symptoms of vitamin A deficiency, although the liver stores were not significantly different from those of the animals receiving 20 units of vitamin A, leads one to the conclusion that the needs of the body tissues which must be satisfied before there is any excess vitamin A available for liver storage must be of considerable magnitude. It also suggests the possibility that lack of vitamin A may effect certain bodily changes, as yet undiscovered, which would serve as a still more

sensitive indicator of vitamin A deficiency than those we rely upon at present. With this idea in mind the brains, spinal cords, and some of the larger nerves of rats from each group were stained by the Marchi technique and examined for signs of degeneration, following the work of Wolbach and Bessey (6). However, no such signs appeared in any of the animals which had either been raised on the low levels of vitamin A or depleted after they were adult. Extensive degeneration, as described by these authors, could be demonstrated in rats depleted to the point of declining weight during the time just after weaning by a diet entirely devoid of vitamin A, i.e., as for bioassay.

#### CONCLUSIONS

Groups of weanling albino rats fed a vitamin A-deficient diet supplemented with 10, 15, 20, 30, 50, and 80 units of vitamin A per kilogram body weight, respectively, give evidence that growth is inhibited in both males and females at the two lowest levels. During the 9 weeks immediately following weaning, females show further significant improvement in growth rate when the intake is raised from 30 to 50 and from 50 to 80 units per kilogram, while males show a significant difference only when the intake is raised to the 80-unit level. In contrast, during the next period the females show no significant difference while the males give evidence of an enhanced growth rate at the higher levels of dietary vitamin A.

The scotopic visual threshold of normal albino rats, as determined by the brightness discrimination test used, lies between log 2.6 to 3.5  $\mu$  lamberts. Hemeralopia appears as the first detectable sign of vitamin A deficiency, followed closely by continuous cornification of the vaginal contents of the females.

Female animals appear to deplete more rapidly than do the males in spite of larger liver reserves, and the vitamin A stores of the liver are practically exhausted before hemeralopia appears.

No quantitatively measurable stores of vitamin A can be demonstrated in the livers of rats fed less than 50 to 80 units of vitamin A per kilogram body weight per day. This is about four times the amount usually considered as the "minimum" requirement for the rat. Judging from the times necessary to produce the usual physiological signs of vitamin A depletion in the 4 groups of animals receiving vitamin A at 4 levels, it appears that some considerable need of the body must be met in an apparently normal animal before liver storage occurs. It also appears as a possibility that more sensitive physiological indicators of vitamin A deficiency remain to be discovered, and that the "minimum" vitamin A requirement may be somewhat higher than 20 units per kilogram body weight per day.

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# THE EFFECT OF CYCLE-LENGTH CHANGES UPON THE FORM AND AMPLITUDE OF THE T DEFLECTION OF THE ELECTROCARDIOGRAM

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One of the best-known facts with respect to the electrocardiogram is that the T wave in one and the same person is likely to change in form and amplitude from time to time. For the most part, the mechanisms by which the several factors or conditions, which may be associated with these changes, produce their effects are not understood. In fact, among the several factors, only the effect of local myocardial ischemia or anoxia and of changes in cycle length can even be interpreted in terms of the local effects which they exert upon the electrical changes in the muscle. And in the application of these explanations there still remain a large number of questions to which no answers can be given. We are dealing simply with phenomena, not with explanations in terms of the relationship between physical and chemical processes and the observed phenomena. We can assert, with reasonable assurance, that the T-wave changes induced by local myocardial ischemia in the mammal are due to the relative slowness with which the ischemic muscle usually becomes repolarized (1, 2). That there is a relationship between the slow repolarization and a delay in the completion of chemical recovery seems probable, but the relationship has not been elucidated. We also feel confident that the relationship between heart rate or cycle length and the rate at which the muscle becomes repolarized after its response begins is related to the form and amplitude of the T wave. Again, we may be sure that some relationship exists between the electrical and the chemical phenomena, but the nature of this relationship is wholly unknown. This paper does not attempt to explain that relationship. Its purpose is to demonstrate that the known facts with respect to the effect of cycle-length upon the time-course of repolarization of cardiac muscle enable us to interpret, in such terms, the effect of cycle-length changes upon the form and amplitude of the T wave of the electrocardiogram.

The fact that rate changes may affect the duration of mechanical systole, of the Q-T interval or its equivalent, and of the absolute refractory period of the ventricles, has long been known, and frequently described. Lombard and Cope early pointed out the inverse relationship between heart rate and the duration of systole (3). Fredericia (4), Bazett (5) and Ashman (6), among others, have proposed empirical formulae relating cycle length and the Q-T interval; while the relationship between the durations of mechanical systole and of the electrical change has been studied, notably by Blair, Wedd and Young (7). Bazett made the important observation that in sinus arrhythmia, the

duration of the Q-T interval does not immediately change from that characteristic of one cycle length to that characteristic of another cycle length, but that there is a progressive or cumulative change. It is this fact, one which must be referred to again, which explains the absence of easily measurable changes in the T wave of the post-extrasystolic beat in normal persons.

In order to understand the relationship between the Q-T interval and the form and amplitude of the T wave, it is necessary to recall a number of theoretical considerations, some of which have been set forth in more detail elsewhere. The T wave is associated with the repolarization of the ventricular muscle, which was depolarized during the inscription of the QRS group of deflections on the electrocardiographic film. If the time required for depolarization is the same for each muscle element, and the time-course of repolarization is also everywhere the same, the net area of the QRS complex, above or below the base line, will be equal to the net area of the T wave, but their directions will be opposite (8). This statement neglects changes in position of the muscle elements during systole. For purposes of reference, we may refer to the T wave from a muscle which is uniform in respect to the time-course of its repolarization as T'. Confusion is avoided if we think of this T' wave as if it were always present.

Very few human electrocardiograms reveal QRS complexes and T' waves which are equal in net area but opposite in direction. Commonly, the QRS and the T wave have the same direction, and there is no consistent relationship between their areas. The most probable explanation of this fact is that the rate of repolarization is more rapid in some muscle elements, and slower in others. In general there is reason to believe that the subepicardial muscle laminae normally become repolarized more rapidly and that the subendocardial laminae are slower (8, 9); but this localization of the rate differences is not essential to our argument. All we are concerned with is that differences exist, and that these differences are responsible for the difference between the T' wave of a physiologically uniform heart and those T waves which are actually observed.

**EXPERIMENTAL.** A. In one series of experiments on three hearts, the excised, quiescent, turtle ventricle was placed upon a moist filter paper on the bottom of an inverted watch crystal. Needle electrodes led the stimulating current from the secondary coil of an inductorium to the ventral, left basal, region of the ventricle. By way of Zn-ZnSO<sub>4</sub> non-polarizable electrodes, current was led from the right base and apex. The heart was brought into connection with the "boot" electrodes by means of absorbent cotton wicks wet with Ringer's solution. The apical wick was tied to the frenum to prevent slipping. Records were obtained by means of a Cambridge Mobile All-Electric Electrocardiograph. In the first series of experiments the ventricle was stimulated several times at each of the following intervals: 20 sec.; 10 sec.; 7.5 sec.; 5 sec.; and then, somewhat irregularly, at shorter intervals to the highest rate at which it would respond. After a 30 sec. rest, another response was usually recorded. After a few minutes' rest, the experiment was repeated, but Ringer's solution, about 12 to 15°C below room temperature, was allowed to drip upon the apex, the

curvature of the watch crystal serving to drain it away from the rest of the heart. Examples of the results are shown in figure 1.

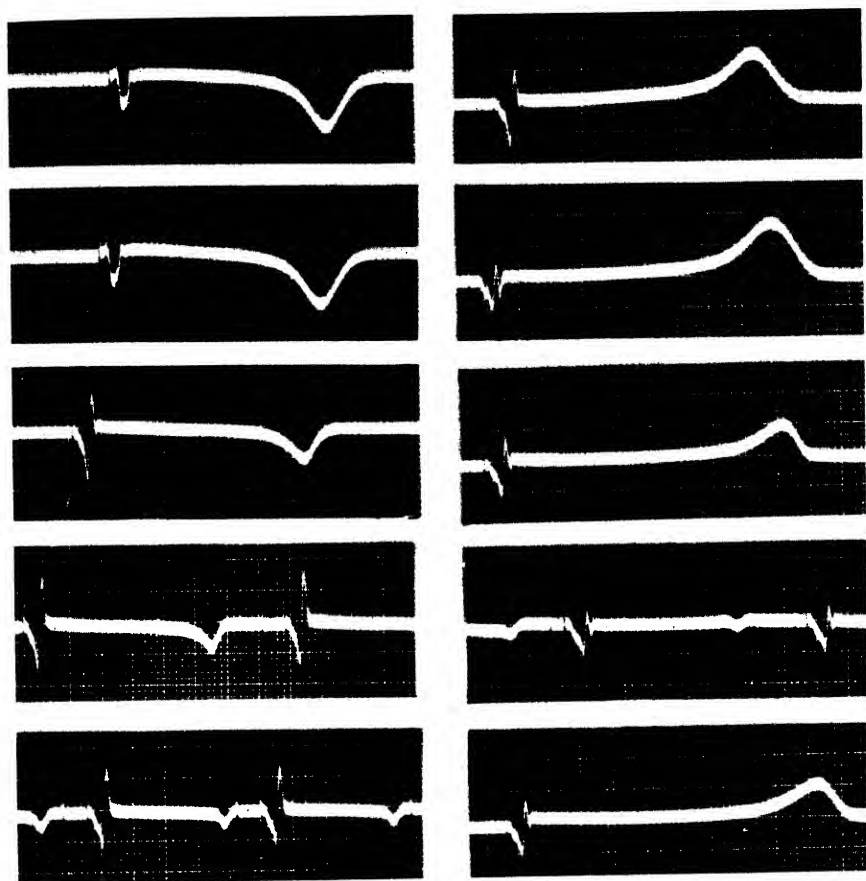


Fig. 1. On left, from above downward, electrograms from base to apex of an excised turtle ventricle. The upper strip shows a wide, inverted T wave. This demonstrates that repolarization at the base was slower than at the apex. The cycle lengths, from above downward, were 20.00, 7.50, 4.00, 1.65 and 1.08 sec. At the shortest cycle length the "Q-T" interval is greatly shortened, and the base becomes repolarized only slightly more slowly than the apex.

On the right, the apex of the same heart was cooled to about  $10^{\circ}\text{C}$  below the room temperature. The repolarization of the apex is now slower than the repolarization of the uncooled base. The cycles, from above downward, were 20.00, 10.00, 4.00, 1.50 and 20.00 sec. The "Q-T" at 10.0 sec. is longer than at 20.0 sec. because the apex was cooler. The lowest curve was recorded 20.0 sec. after the one just above it to demonstrate that the apex was still cool. Note that at cycle 1.50 sec., the apex becomes repolarized more rapidly than the base, in spite of continued cooling.

*B.* In other experiments on four hearts, the whole heart was immersed in an evaporating dish. One electrode was a suction electrode, in principle like that described by H. C. Wiggers (10). This, after recording a few normal



responses without suction, was attached to the ventral heart surface for the recording of monophasic responses. The other electrode dipped into the solution several centimeters from the heart. With the solution at room temperature, a series of monophasic curves were recorded at various rates, as described in A, but more care was exercised in controlling the rates, and slower and faster series were alternated to minimize cumulative changes. The experiment was then repeated, after a few minutes, when the solution was about  $10^{\circ}$  to  $20^{\circ}$  below the room temperature. Sample curves from these experiments are shown in figure 2.

C. In several experiments, with arrangements as in A, the heart was stimulated at a basic rate, varying from 6 to 20 per minute in different trials, premature beats, followed by "compensatory pauses", occasionally being elicited. In some experiments the apex was cooled; in others it was not cooled. In several experiments the base was injured by cutting.

RESULTS. *Procedure A.* In figure 1 are shown the curves, derived from 2 points on the ventricular surface, and recorded at room temperature. In the upper strip on the left the cycle was 20 sec. A fairly large T wave is present, indicating that the rate of repolarization of the right basal surface in this case was slower than the rate at the apex. The second curve on the left was recorded when the cycles were 7.5 sec.; the third, when they were 4.0 sec.; the fourth, when they were 1.65 sec. The lowermost strip on the left shows a much higher rate, the average cycle being a little over a second. The T wave is now very small, although the repolarization at the base is still slightly slower than at the apex. With respect to the two points from which the curves were derived, the rates of repolarization are more nearly equal when the cycles are short than when they are long.

This was the consistent result in all experiments.

In figure 1 on the right, the apex of the same heart has been cooled. Because of the cooling (uppermost strip) the rate of repolarization at the apex is slower than at the right base, and the Q-T interval is longer than in the experiment at room temperature. The second strip shows a 10 sec. cycle. Cooling had been renewed, so that the Q-T is slightly longer than before. The third strip, at 4 sec. cycles, shows a lower T wave. At the high rates (strip 4), despite continued cooling of the apex, the T waves are very low and actually inverted in this case, and the Q-T interval is apparently as short as it was in the experiment at room temperature. The lowermost curve is a control, without renewed cooling, taken 20 sec. after the fourth strip to prove that the apex was actually cool when the fourth strip was recorded. It is again evident in this experiment that, with respect to the two points from which the curves were derived, the rapid rate has caused the time-courses of repolarization to become nearly the same. This phenomenon has been noted by Ashman and Hull (p. 40) (11).

These results are typical of the whole series. They are not intended to be quantitative, but merely qualitative. They demonstrate, in striking fashion, the phenomenon seen in the normal human electrocardiogram when great rate changes occur. An example, from a human patient without heart disease, is

shown in figure 3, middle and lower strips, from lead II. The lower strip was taken during a paroxysmal tachycardia.

*Procedure B.* Figure 2 shows sample monophasic curves from the heart at two different temperatures. On the left in the figure, from above downward, are shown three sample curves from the ventricle, cooled to  $20^{\circ}\text{C}$  below room temperature, at cycles of 20, 7.5 and 4 sec., respectively. On the right curves from the same ventricle at the same cycle lengths are shown, but the temperature was nearly  $10^{\circ}\text{C}$  higher. It will be seen that when the cycles are long, the curve from the cooled heart has a much greater duration than the curve from the same heart, uncooled. When the cycle is short, the curves have practically the same durations. The use of monophasic curves made it possible to measure with reasonable

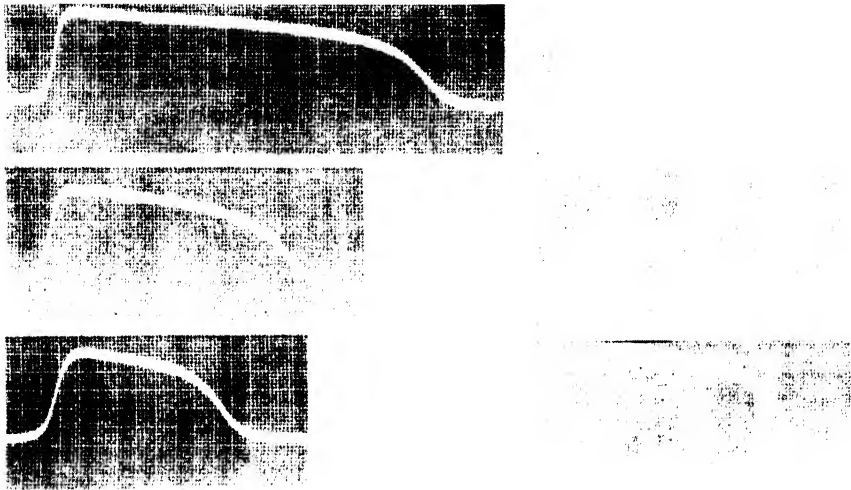


Fig. 2. On the left, monophasic curves from the immersed ventricle at about  $20^{\circ}\text{C}$ . below room temperature. On the right, from the same heart, the temperature was about  $10^{\circ}\text{C}$  below room temperature. In both series, the cycles were, from above downward, 20.0, 7.5 and 4.0 sec. Note that at the 20.0 sec. cycles, the monophasic curve of the ventricle when colder exceeds 4.2 sec., when warmer, 2.7 sec. When the cycles are 4.0 sec., the durations are practically equal at 1.90 sec. The full series from this heart is shown in figure 4. The heights of the curves are irrelevant.

accuracy the time required for repolarization of membranes near the tip of the suction electrode. The theory need not detain us. The measurement was made from the end of the rising limb of the curve to its end. In experiments by this method, the whole heart was cooled; we are, therefore, comparing the effect of cycle length upon the time course of repolarization in the cooled and relatively uncooled heart. Since cooling lengthens the absolute refractory period of the muscle, it was impossible to drive the cool ventricle to as high a rate as the uncooled.

In figure 4 the duration of the monophasic curve (ordinates) is plotted against the cycle length (abscissae). In this case, three temperatures were used. At a cycle length of 2 sec., which marks the approximate end of the effective re-

fractory period of the cooled ventricle, the durations of the monophasic curves of both the moderately cooled (curve *b*) and the same muscle at room temperature (curve *a*) are nearly the same. This, of course, is also what is shown in figure 1,

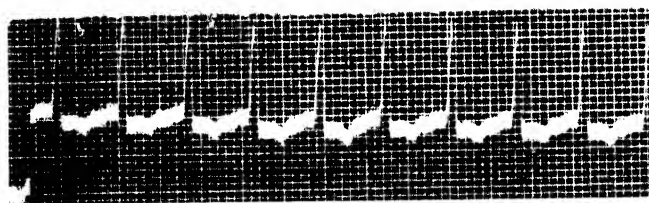
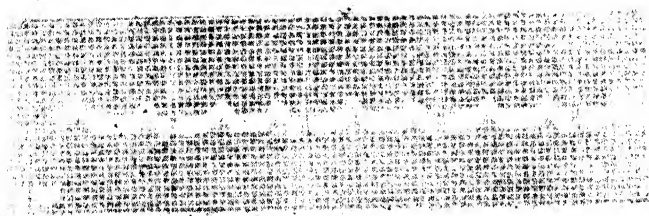
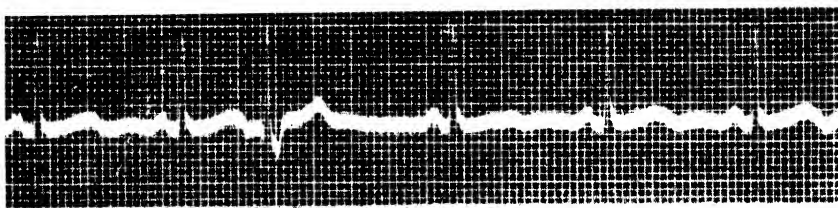


Fig. 3

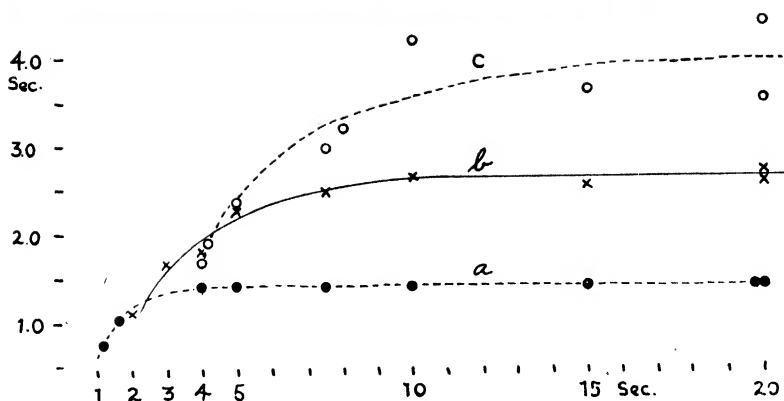


Fig. 4

by a different method. As the cycles become longer, the curves deviate more and more, so that, at 20 sec., the monophasic curve of the moderately cool muscle has approximately double the duration of the curve from the uncooled muscle. When the muscle was 20°C below room temperature (curve *c*) there is much more scattering of points, due in part to cumulative changes and in part to a

rise in temperature of the solution in which the muscle was immersed. Yet the trend is unmistakable.

It is true, as Blair, Wedd and Young (7) have pointed out, though not for the first time, that if the heart be driven at a high rate for a time, the electrical responses become progressively shorter. This may also happen when a change of rate occurs abruptly in the human heart, but in this case it is obvious that an equilibrium, at a new Q-T duration, is established sooner or later. To minimize cumulative changes in our experiments, we have stimulated only 5 or 6 times at each rate, with pauses intervening.

*Procedure C.* This is essentially the same experiment as method A, but adopted in imitation of premature beats and compensatory pauses. Because of slow recovery after cumulative changes, the T wave following the compensatory pause was usually not strikingly different from the other T waves; yet in a number of experiments, after injury or cooling, T-wave changes were quite clear.

Figure 3 shows the phenomenon in a human heart. The upper strip is a sample of lead II from a patient with myocardial disease. The T waves of the regular beats differ considerably from those of the post-extrasystolic beat. There is a lengthening, as we interpret it, of the duration of repolarization of certain muscle surfaces relative to others. This phenomenon is often observed when parts of the muscle are ischemic, although ischemia may not be the only cause. Evidence, which need not be given here, indicates that often the subepicardial lamina in the affected heart region suffers more than the immediately subendocardial fibers (1). Following longer cycles, the ischemic muscle apparently becomes repolarized more slowly than the non-ischemic muscle; but after shorter cycles, the rates in the two become more nearly equal, or the normal relationship of more rapid repolarization of subepicardial fibers may even be seen. Ashman and Hull have earlier offered this explanation for the changes in the human electrocardiogram (legend of their fig. 121, p. 328), but experimental support was lacking (11). This observation suggests that a comparison of the T waves after carotid sinus pressure with those after cardiac acceleration may yield better diagnostic evidence than a change in rate in one direction alone.

**DISCUSSION AND SUMMARY.** A proper discussion of the T wave of the electrocardiogram necessarily involves consideration of the ventricular gradient of F. N. Wilson (8). Since a full discussion, in terms of the gradient, cannot be given briefly, we shall simply give the references here (8, 12, 13, 14). When the time course of repolarization of all the muscle elements of the heart is the same, we then find the QRS complex to be followed by the wave we have designated above as T'. It is the electrical effect of a "wave" of repolarization passing through the muscle along the same pathways, and with the same velocity, as the earlier wave of depolarization or excitation. Those muscle elements which are first to become depolarized are first to begin, and first to complete, their repolarization in the physiologically homogeneous muscle. No matter what the actual time course of repolarization in different regions may be, the elements first to be activated are, during the period of inscription of the T wave, *rel-*

*actively* farther advanced temporally in repolarization than those elements which are last to be activated. This produces an electrical effect, normally always present, which is, in effect,  $T'$ . The T wave which is usually actually observed is the net electrical effect of differences in the time course of repolarization of different muscle elements, plus  $T'$ .

Our experiments have demonstrated:

a. That when the turtle heart is beating slowly, the time courses of repolarization at the base and apex may differ. By cooling the apex (or base) greater differences in "rates" of repolarization may be induced, as is well known, with corresponding modifications in the T wave.

b. When the heart is driven rapidly, either the spontaneously occurring difference or the artificially induced difference in time course of repolarization can be practically eliminated.

c. Corresponding to this elimination of differences in "rates" of repolarization, the T wave assumes, or nearly assumes, the  $T'$  form.

d. Similar changes, seen in the human heart in association with changes in cycle-length, are attributed to the same cause. It is assumed that the T wave commonly observed in the human electrocardiogram is due predominantly to differences in time course of repolarization in different muscle elements. A rapid tachycardia, by minimizing or eliminating the differences in regional repolarization rates, changes the T wave from its usual form to a form which approaches the  $T'$  form. The latter varies, depending upon the form and net magnitude of the QRS complex which precedes it.

e. When there is a change in heart rate, there is not ordinarily an immediate adjustment of repolarization rate to the new heart rate. Instead, there is a more or less gradual adjustment until, in the human heart, an equilibrium is attained. When the muscle is depressed, as by anoxia, the immediate changes may be far more pronounced (11). In the normal heart, therefore, a compensatory pause, for example, is usually followed by a T which differs little from the T waves of the regular beats. When the muscle is damaged, on the other hand, very conspicuous changes in the T wave may often be seen (15). The simple effect of the change in the cycle length is not necessarily the only cause of the change in the form and amplitude of the T wave; but it is likely that it is by far the most important one.

f. The higher cardiac rates associated with childhood, fever, or thyrotoxicosis are paralleled by differences in the metabolic rate of the heart muscle. Consequently, the amplitude of the T wave may not differ from the usually observed one (14). In the tachycardia of vigorous muscular exercise also, some factor, perhaps related in an unknown manner to metabolism, to nervous influence, and to cardiac output, partly offsets the effect of rate (14). Acceleration resulting from the inhalation of amyl nitrite is associated with a change in the T wave, of the same magnitude as that due to spontaneous acceleration (14).

g. Spontaneously occurring rate changes in the human heart are experiments provided by nature. It is unlikely that mammalian experiments, directed to the study of this particular problem, would afford more reliable information

than the human electrocardiogram. It may here be noted, however, without attempting to give the full, long argument, that experiments purporting to explain the human T wave as an effect of a greater duration of right than of left ventricular response are almost irrelevant to the problem. What little evidence exists suggests that the differential time courses are of subepicardial and subendocardial laminae, as has been suggested. Yet this suggestion explains little. There must be differences in rates of repolarization between different parts of single muscle fibers or between closely adjoining portions of small syncytical masses; and these effects summate to give the T wave.

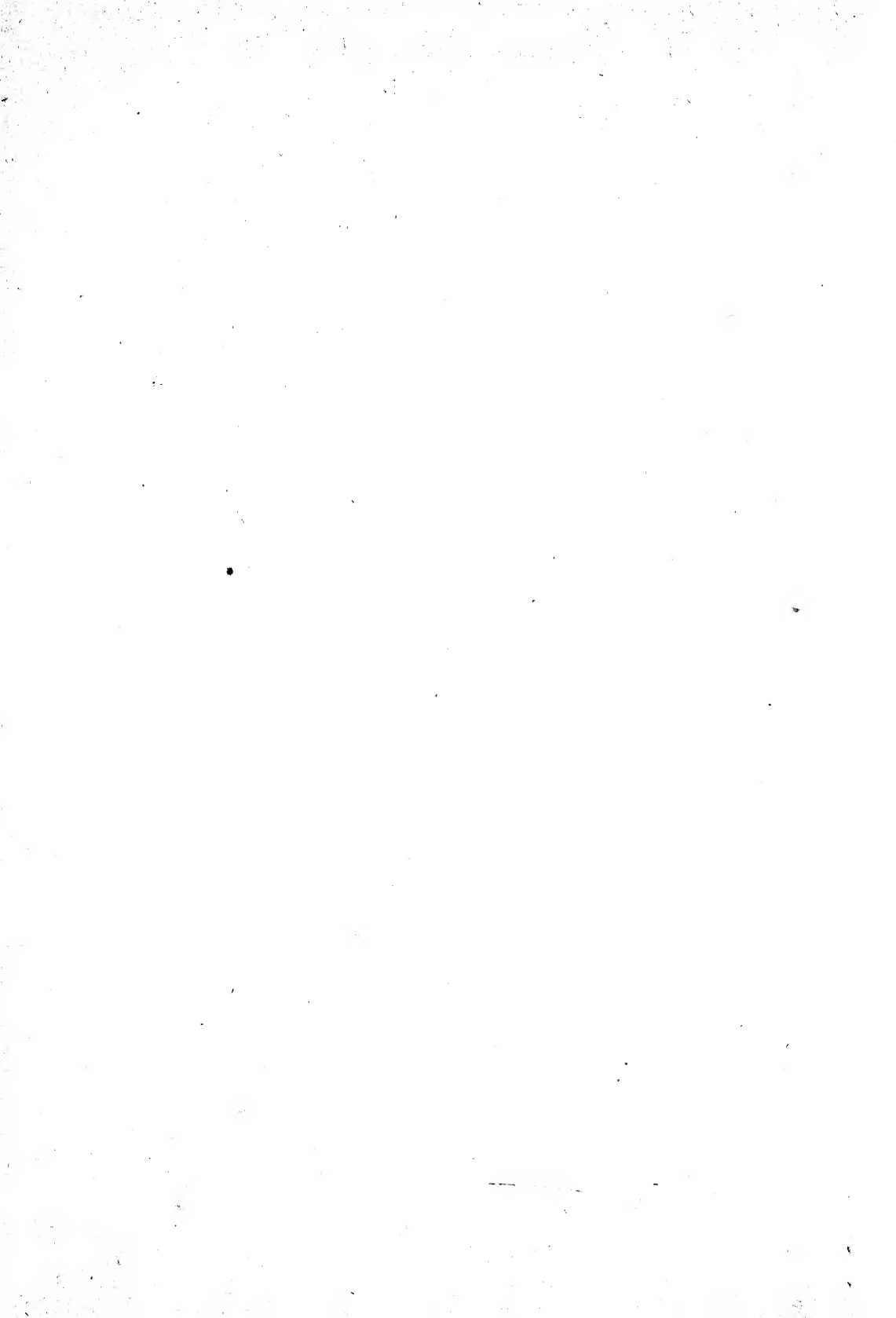
Of particular physiological interest is another implication of these experiments, namely, that the T wave is wholly, or almost wholly, an electrical consequence of the repolarization of the cardiac muscle. This is evidently true both when the T wave has its usual form and direction and when, because of rapid heart rate, digitalization, or pathological change, the T wave is of the T' form. These experiments also serve to emphasize another point which has often been overlooked. This is that attempts to determine the metabolic or other effect of certain agents upon the heart muscle from a study of the electrocardiogram of the intact animal is almost futile. On the other hand, a study of the effects of these agents upon the monophasic action current curve may prove to be a fruitful and valid method. When these latter effects are established, it may then be possible to approach the explanation of their relationship to the form of the T wave of the electrocardiogram.

#### CONCLUSIONS

In the normal heart, at ordinary heart rates, the observed T wave is mainly due to differences in the time required for repolarization of different muscle elements. Acceleration, or decrease of cycle length, reduces these differences and thus causes the associated changes which then occur in the form and amplitude of the T wave.

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## THE DYNAMICS OF THE ISOLATED HEART AND HEART-LUNG PREPARATIONS OF THE DOG<sup>1</sup>

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Considerable work has been done with isolated heart and heart-lung preparations but no systematic attempt has been made, as far as we know, to interrelate the changes of the various pressure levels and rates of flow when one or more of the controllable factors are altered. Lack of appreciation of these interlocking effects has doubtless contributed to erroneous interpretations of data and may have helped to produce some of the polemics.

For the past few years the dynamics of the mammalian heart have been studied in this laboratory in isolated heart and heart-lung preparations. Such preparations were developed with the object of controlling as many variables as possible. Considerable data concerning the relationships of various measurements on these preparations have been accumulated. In this report an analysis of the data obtained soon after the preparations were under control is presented to clarify the dynamics of such preparations and to throw further light on the factors controlling the circulation in the intact animal.

**METHODS, PREPARATIONS AND PROCEDURES.** Dogs mostly weighing from 10 to 20 kilos were used for the isolated heart and heart-lung preparations. In the earlier experiments, sodium pentobarbital (25–37.5 mgm./kilo, in a 2½ per cent solution) was used; later, ether was employed until the head circulation was interrupted since anesthesia is not necessary after this stage. The effect of anesthesia on the completed preparation could thus be minimized. In all instances the chest was opened widely, artificial respiration was instituted, and the azygos vein, superior vena cava, innominate artery and left subclavian artery were doubly ligated and cut between ligatures. This eliminated the head and upper limb circulation; during this period it was often necessary to section the vagi in the neck to avoid the deleterious slowing or standstill of the heart which accompanied the agonal asphyxia of the head. The blood of the

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dog was then rendered incoagulable with intravenous heparin (8–12 mgm./kilo of the Connaught laboratories preparation, or  $1\frac{1}{2}$ –2 cc.-of Liquaemin, Roche Organon, Inc.<sup>4</sup> per dog).

While these procedures were carried out on the animal, the perfusion arrangement was assembled. This consisted of glass and rubber which had been "seasoned" for some time by perfusion with blood. Between experiments it had been washed and was usually kept immersed in water. While the equipment was sterilized with alcohol in early experiments, this was later abandoned as unnecessary. After the perfusion arrangement was assembled it was flushed with isotonic saline and then filled with either defibrinated or, in later experiments, heparinized blood. In preparing defibrinated blood, usually 2 or 3 large dogs were bled, under ether, into large flasks containing glass beads. The flasks were gently rotated and the blood then filtered through fine cambric cloth (50  $\mu$  mesh) from 2 to 5 times to remove traces of fibrin. Finally the blood was passed two to four times through the inflated lungs of a dead dog to remove vasoconstrictor activity otherwise present. Heparin (40–100 mgm./liter) or Liquaemin (0.5–1.8 cc./liter) was added to the defibrinated blood before placing it in the perfusion arrangement.

Later, in the heart-lung preparation, defibrination was omitted and a larger dose of heparin employed. This was found to give a better and longer surviving preparation and to be less prone to lead to pulmonary edema. The only disadvantage was that the heparinized blood in the flowmeters and reservoirs tended to settle out rapidly. The blood was collected as before, each bleeder dog receiving  $1\frac{1}{2}$  cc. of Liquaemin intravenously as a preliminary, and in addition about  $1\frac{1}{4}$  cc. of Liquaemin per liter of blood was added to the flasks. The blood was then filtered through the lungs of a dead dog and then through cambric. Before using the blood a further 1 cc./liter of Liquaemin was added. This was found adequate to prevent coagulation in experiments lasting as long as 8 hours.

It was observed in preliminary experiments that the heart was maintained better when glucose ( $\frac{1}{4}$  to 1 gram/liter of blood) and  $1\frac{1}{2}$  to  $5\frac{1}{2}$  cc. of 10 per cent calcium gluconate per liter of blood (Sandoz<sup>5</sup>) were added. This was, therefore, routine procedure in the early experiments. In a few experiments, NaCl, in quantities of 0.45 to 0.80 gram/liter of blood, was also added. Further details of these precautions can be obtained from our earlier publications (1, 2, 3, 4).

Two types of preparations were used for our studies.

One was the isolated heart preparation described previously (2 and 3). In the early experiments the coronary sinus was cannulated, in later ones it was not. The total pulmonary arterial flow was measured in all experiments. In the isolated heart preparation the perfusing blood enters the left auricle from a reservoir under pressure which can be varied. An artificial systemic resistance, which can be varied, is interposed between the aorta and the right auricle. The blood from the right ventricle passes through another artificial resistance, which

<sup>4</sup> We are grateful to Roche Organon, Inc. for furnishing us with generous supplies of Liquaemin.

<sup>5</sup> We are indebted to the Sandoz Chemical Works, Inc. for supplying us with the calcium gluconate.

can also be varied, and into an artificial lung for aeration. A pump forces the blood from this "lung" back into the original reservoir. The dynamic state of the preparation can be controlled by adjusting (1) the artificial resistances in the systemic and pulmonary circuits, and (2) the pressure of the blood entering

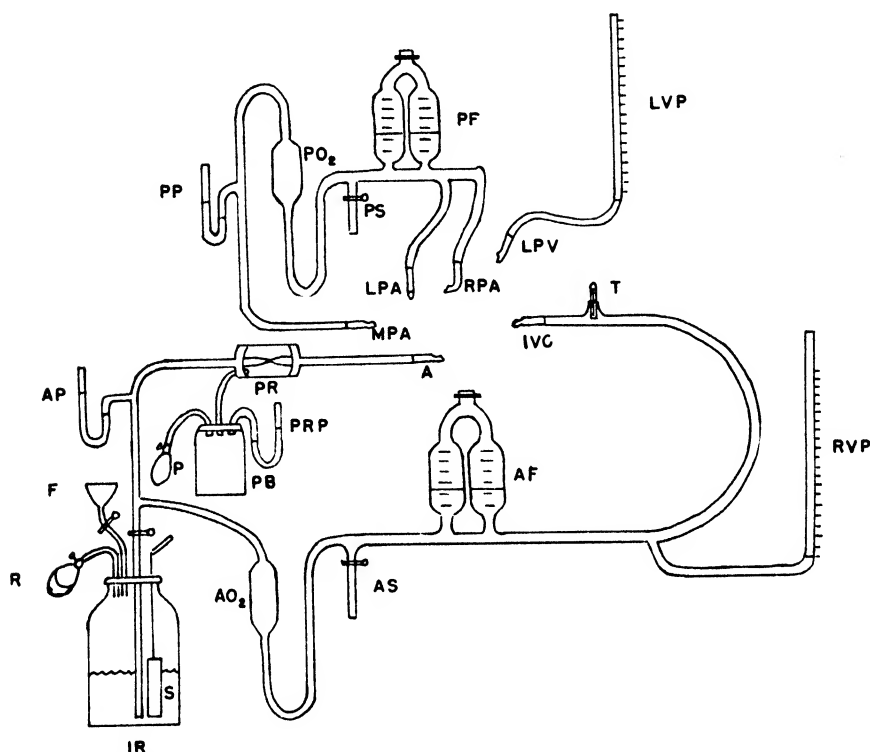


Fig. 1. Schematic diagram of the closed circuit heart-lung preparation described in text. *PP* is the pulmonary pressure manometer, *PO<sub>2</sub>* is the pulmonary arterial *O<sub>2</sub>* level photoelectric meter unit, *PF* is the pulmonary flowmeter, *PS* is the pulmonary arterial sampling tube, *MPA*, *LPA* and *RPA* are respectively the main, left and right pulmonary arterial cannulae, *LVP* is the left venous pressure manometer, *LPV* is the pulmonary vein cannula, *A* is the aortic cannula, *PR* is the peripheral resistance, *P*, *PB* and *PRP* are respectively the peripheral resistance pressure bulb, bottle and manometer, *AP* is the aortic pressure manometer, *IR* is the blood reservoir bottle, *F*, *R* and *S* are respectively the filling funnel, the pressure bulb and shaker for the blood reservoir bottle, *AO<sub>2</sub>* is the aortic *O<sub>2</sub>* level photoelectric meter unit, *AS* is the arterial sampling tube, *AF* is the aortic flowmeter, *RVP* is the right venous pressure manometer, *IVC* is the inferior vena cava cannula and *T* is a thermometer.

the left heart from the reservoir. In this preparation, the output of the left ventricle determines the output of the right.

The second type of preparation, the closed circuit heart-lung preparation, has also been described previously (4 and 5). In figure 1 is presented a simplified diagram of the latest arrangement which differs only in non-essential details from the earlier ones employed. It differs from the usual heart-lung preparation

in having a blood reservoir which is not a part of the circuit but is available to increase or to decrease the circulating blood volume. In this preparation, therefore, the control of the dynamic state of the circulation is achieved by varying the amount of blood in circulation and the amount of resistance in the artificial systemic resistance.

While the details of the operative procedures have been presented in previous communications for the isolated heart preparation (2 and 3), this has not been done for the closed circuit heart-lung preparation. In brief, the procedure after the chest is opened is as follows:

After the head circulation has been interrupted by tying the innominate and left subclavian arteries and the azygos vein and superior vena cava, double heavy ligatures are placed around the root of the aorta, the main pulmonary artery, the major right and left pulmonary arteries and the inferior vena cava. A single ligature is passed about the descending aorta near the arch. These are all left untied.

The thoracic descending aorta is then tied off near the arch and simultaneously the thoracic inferior vena cava is clamped at the diaphragm with a rubber covered hemostat. The latter procedure permits use of the blood in the abdomen to replace blood loss in the succeeding operations. This we have found to be an important step which often spells the difference between success and failure. At this stage, the peripheral systemic circuit consists solely of the coronary vessels.

The next step is the cannulation of the ascending aorta, care being taken that the cannula is above the semilunar valves; before cannulation, the artificial resistance is set at a pressure of about 100 mm. Hg. By adding blood slowly from the abdomen through the inferior vena cava, the aortic pressure can be built up to around 100 mm. Hg. Sufficient time is allowed before proceeding with the next step to permit the heart to become adjusted and regain a vigorous beat. The insertion of the inferior vena cava cannula then completes the heart-lung circuit. A period of some 15 minutes is again allowed for adaptation of the heart to the new circumstances. By carefully varying the peripheral resistance and the blood volume in the preparation, the heart can be maintained in an adequate state. The blood in the reservoir must be thoroughly agitated before additions to the preparation are made. During this period a cannula is inserted into a small branch of the left pulmonary vein near the lung hilus, facing the heart, to record the left venous pressure.

The heart-lung preparation is now removed from the animal and placed in a glass tray. In order to measure the total pulmonary blood flow and the pulmonary arterial pressure, a flowmeter and a T-tube for connection with a mercury manometer are inserted, by means of glass cannulae, between the main pulmonary artery and its two branches. The order of cannulation is: 1, right pulmonary artery distally; 2, main pulmonary artery centrally (immediately establishing the circuit between them), and 3, left pulmonary artery distally. The cannulae are adjusted to avoid kinking and the whole preparation in its tray is enclosed in a constant temperature box with appropriate windows and doors for inspection and adjustments, and the preparation is ready for experimental study.

The methods of taking readings, controlling conditions, and making the measurements and calculations have been described (2, 3, 4).

The pertinent data on 44 isolated heart and 35 heart-lung preparations are summarized in tables 1 and 2. The control levels given are either initial readings made after attaining stabilized conditions at the desired levels of flows and pressures, or, where conditions could not be stabilized, readings early in the experiment, coincident with the taking of the first oxygen sample. The criteria used to judge the onset of progressive heart failure or its initial presence will be described in another communication (6). By total duration of the experiment is meant the time during which readings were taken. Experiments were terminated by severe heart failure, by accident, or by some severe arrhythmia (as occurred chiefly in the preparations to which digitalis was added).

A total of 119 graphs were made of the data in tables 1 and 2. The scatter graphs were subjected to statistical analysis to determine whether significant linear correlations were present. The statistical data so obtained are shown in table 3. All surviving preparations are included in this analysis. Consequently, initial readings were made upon hearts in very different biological conditions. These 79 preparations varied in the type of circuit, in the weight of the dog and heart, in the duration of time required to establish the perfusion set-up, etc. In view of the expected variations from heart to heart it appears all the more significant that the control levels of pressures and flows and their interrelations showed certain trends and correlations. Absence of statistically demonstrable linear relationship between two variables may mean 1, that none exists; 2, that the data are insufficient to demonstrate an expected correlation, or 3, that the relationship is curvilinear. The interrelationship of those variables involving coronary flow will be considered in another communication (8). The others will be discussed below.

*Duration of Experiment, Time of Onset of Progressive Failure and Duration of Progressive Failure.* These time periods were quite variable as can be seen in columns 5 and 6 of tables 1 and 2, and from the differences between the corresponding figures of these two columns which give the duration of progressive heart failure. It will be seen that the onset of progressive heart failure tended to occur later, its duration and the duration of the experiment tended on the average to be longer in the heart-lung than in the isolated heart. In the heart-lung preparation these times tended to be longer when the donor blood was heparinized than when it was defibrinated. Apparently, the artificial lung used to aerate blood in the isolated heart preparation leads to a more rapid change in the blood, probably an accelerated destruction of the red cells which releases materials detrimental to the heart (possibly potassium, the detrimental action of which is known (7 and 9)). This also obtains when blood is defibrinated since in this process considerable agitation of blood is essential.

No correlation of these time values could be demonstrated with the amount of work being done by the heart, the temperature of the preparation, or the oxygen level in the arterial blood perfusing the heart. The possibility that these three variables interplayed in determining these time periods was examined by plotting

TABLE 1  
Control values in isolated heart preparations

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
EXP. NO.	MATERIAL ADDED TO PERFUSATE	BLOOD TEMP.	DEGREE OF PROGRESSIVE HEART FAILURE AT START	TIME OF ONSET OF PROGRESSIVE HEART FAILURE	TOTAL DURATION OF EXP.	HW (AT END OF EXP.)	AP	PP	LVP	RVP	PF	AF	CF	HR	W	ART. O <sub>2</sub>
		°C.		min.	min.	grams	mm. Hg	mm. Hg	cm. H <sub>2</sub> O	cm. H <sub>2</sub> O	cc./min.	cc./min.	cc./min.	beats/min.	kgM./hr.	vol. %
R <sub>1</sub>	CG	35.0	0		54	94.5	95	11	8.5	1.6	429	288	161	124	37.2	13.2
1		35.5	0	10	47	94.4	102	6	7.6	0.6	224	128	96	104	18.9	10.5
2		37.0	0	2	16	78.3	101	3	13.0	27.0	209	129	80	88	17.6	8.0
4		34.5	0	30	151	148.0	95	3	7.5	3.5	283	152	131	140	22.3	14.0
5		35.2	0	80	186	155.0	96	6	11.5	3.5	227	144	83	84	20.4	11.5
7		34.7	0	92	161	126.0	94	18.5	6.6	4.8	395	312	83	88	34.5	7.0
10		35.0	0	10	70	125.0	90	4	9.3	3.5	207	120	87	94	15.4	13.0
11	CG	33.5	0	10	113		93	5	8.5	2.5	263	120	143	100	21.1	
12	CG	34.0	0	20	141	168.0	92	15	6.8	2.5	349	138	211	108	30.5	9.4
14	CG	35.0	0	90	133		99	15	10.2	3.9	428	341	87	116	39.4	13.5
R <sub>2</sub>			0	8	31	184.5	95	24	6.3	3.3	441	170	271	132	44.9	10.9
15a		36.0	0	72	116	190.0	94	14	5.5	3.0	750	358	392	132	66.0	11.6
17	C	35.0	0	10	50	83.0	99	16	10.0	0.7	297	224	75	112	28.0	13.5
20	C	37.0	0	3	26	88.2	94	12	9.3	9.6	242	190	52	90	20.9	11.6
21	CG	36.0	0	16	51	124.1	102	18	17.1	10.0	263	200	63	102	25.8	12.6
40	CG	33.5	0		54	57.0	98	17	12.3	21.0	405	333	72	96	38.0	16.0
41	CG	31.0	0	75	143	83.2	104	13	4.7	7.9	313	146	167	92	29.9	14.6
44	CG	31.0	0	95	168	136.5	93	20	6.0	14.3	176	100	76	74	16.2	19.5
45	CG	29.0	0	35	79	51.0	98	21	5.4	5.2	177	132	45	50	17.2	20.0
46	CG	29.5	0		120	95.0	97	20	4.6	3.2	174	114	60	80	16.6	18.8
47	G	30.0	0		112	75.0	100	20	9.7	5.5	171	125	46	80	16.7	19.9
48	CG	29.5	0		98	62.2	105	21	13.8	8.4	149	99	50	84	15.3	19.2
50a	CG	29.0	0		150	141.0	98	26	10.7	3.3	211	33	178	68	21.3	14.5
51	CG	30.5	0	90	91	74.0	87	17	7.5	4.9	116	47	69	92	9.8	10.5
51a	CG	30.0	0	25	52	91.0	84	15	7.0	2.3	104	34	70	126	8.3	16.7
53	G	30.5	0	12	87	101.7	103	23	10.8	5.0	200	142	58	74	20.5	20.1
3		34.0	0	40	47						167	113	54	92		
R <sub>3</sub>	CG	35.0	0	2	12						254	200	54	60		
21A		33.0	0		15						405	366	39	92		
6		34.5	M	0	86	123.0	90	4	9.5	3.5	312	93	219	100	23.9	12.0
9	CG	33.5	M	0	75	91.1	91	8	7.4	3.8	144	82	62	92	11.6	13.0
13	CG	35.5	M	0	83	129.5	98	11	8.8	3.7	417	294	123	132	37.0	11.7
15	CG	35.0	M	0	84	144.0	100	12	2.2	3.5	428	200	228	148	39.2	10.4
16	CG	36.5	M	0	67	140.0	93	9	9.3	4.2	326	219	107	88	27.1	15.7
R <sub>4</sub>	G	34.0	M	0	46	106.0	92	9	9.1	2.3	273	195	78	84	22.5	14.3
18	C	34.0	M	0	130	83.8	96	19	6.1	2.8	341	263	78	80	32.2	13.2
19	C	36.5	M	0	71	121.5	103	11	5.6	1.6	177	123	54	92	15.6	17.9
42	CG	32.5	M	0	87		92	7	4.4	2.2	130	66	64	92	10.5	18.5
43	CG	31.5	M	0	83		94	15	7.1	8.7	176	125	51	102	15.5	20.8
49	G	30.0	M	0	193	142.8	100	22	3.4	6.4	144	87	57	72	14.2	19.6
50	CG	29.0	M	0	157	99.0	104	19	6.0	9.1	163	102	61	88	16.3	17.5
52	G	30.6	M	0	126	121.0	93	22	5.8	6.9	98	40	58	76	9.1	16.8
R <sub>4</sub>	CG	35.0	M	0	14						288	133	155	100		
R <sub>5</sub>	CG	37.0	S	0	13						375	175	200	106		

Perfusate in all experiments—defibrinated blood. Recipient dog blood made anticoagulable by use of heparin (see text).

C—calcium gluconate } see text for quantities added to perfusate.

G—glucose

M—mild failure

MS—moderately severe failure

S—severe failure

HW—heart weight

AP—aortic pressure

PP—pulmonary arterial pressure

LVP—left venous pressure (systemic)

RVP—right venous pressure (pulmonary)

PF—pulmonary artery flow

AF—aortic flow

CF—coronary flow

HR—heart rate

W—work of heart

Art. O<sub>2</sub>—O<sub>2</sub> content of arterial blood

their product against each of these time values. No correlation could be established. Apparently the variations in the state of the heart after the preparation was established and the character of the blood were much more

TABLE 2  
*Control values in heart-lung preparations*

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
EXP. NO.	MATERIAL ADDED TO PERFUSATE	BLOOD TEMP.	DEGREE OF PROGRESSIVE HEART FAILURE AT START	TIME OF ONSET OF PROGRESSIVE HEART FAILURE	TOTAL DURATION OF EXP.	HW (AT END OF EXP.)	AP	PP	LVP	RVP	PF	AF	CF	HR	W	ART. O <sub>2</sub>
		°C.		min.	min.	grams	mm. Hg	mm. Hg	cm. H <sub>2</sub> O	cm. H <sub>2</sub> O	cc./min.	cc./min.	cc./min.	beats/min.	kgM./hr.	vol. %
23*	C	34.5	0	100	320		80	12	2.0	1.0	107	74	33	102	7.9	16.5
23a*		30.0	0		25		102	38.5	13.1	9.8	547	357	190	68	62.8	
24*	C	34.5	0	40	98	112.0	88	10	5.0	6.4	205	44	161	72	16.3	14.7
25*	C	35.0	0	130	185	135.0	110	54	8.5	12.1	455	273	182	100	61.0	12.0
26*	C	33.5	0	70	111	111.8	97	39	12.5	7.2	361	259	102	88	40.1	11.8
27*		33.5	0	19	96	129.0	93	15	11.3	4.9	242	143	99	104	21.1	7.8
28*	C	31.0	0	197	215		81	22	6.6	7.8	185	149	36	130	15.1	13.3
29*	C	33.0	0	56	82		103	25	12.4	6.9	254	221	31	94	26.5	13.6
30*	CG	33.0	0		80	92.0	97	25	25.1	8.3	98	67	31	90	9.7	14.5
31*	C	33.0	0	23	161	82.0	93	47	7.0	9.9	195	106	89	144	22.3	22.0
32*	CG	30.0	0	109	128	87.0	97	10	4.1	3.3	105	69	36	84	15.0	16.2
33*	CG	33.0	0		121	48.0	101	15	3.3	6.1	238	158	80	135	22.6	10.4
34*	CG	30.7	0	138	148	98.5	105	19	10.8	30.0	181	146	35	72	24.2	15.9
35*		30.0	0	181	270	65.5	101	19	7.5	10.9	167	137	30	90	16.2	15.9
36*	C	33.0	0		162	80.6	84	26	9.1	39.7	155	107	48	144	13.8	15.8
37*		32.2	0		134		99	27	1.4	1.7	140	100	40	90	14.3	14.2
38*		33.0	0		126	73.5	101	16	4.9	6.8	176	143	33	102	16.7	17.5
39*		33.0	0		124	84.0	91	16	4.8	4.6	158	108	50	108	13.7	15.3
55*		36.0	0	25	163		74	11	2.5	3.5	150	116	34	120	10.5	20.5
59†	CG	38.0	0	6	28		79	21	3.6	1.7	187	74	113	116	15.2	9.0
60†	CG	33.0	0	60	228		92	16	4.8	3.7	217	175	42	68	19.0	
61†	CG	32.0	0	190	253		79	6	0.4	15.6	192	60	132	64	13.1	21.9
62†	CG	34.0	0	104	356		81	4	4.5	13.5	197	120	77	92	13.7	21.0
63†	CG	29.5	0	20	232		102	18	1.9	13.7	484	321	163		47.6	18.0
64†	CG	31.0	0	65	293	148.0	96	18	0.7	4.6	390	278	112	68	36.5	17.5
66†	CG	29.0	0	60	290		66	10	1.3	7.5		80		60		19.7
67†	CG	30.0	0	65	195		94	26	5.1	3.3	211			70	20.7	15.6
68†	CG	31.5	0	160	293	130.0	92	11	5.5	6.0	171	165	6	88	14.4	21.6
22*		31.0	S	0	94		89	15		6.1	217	126	91	68	18.5	7.0
54*		38.0	S	0	26		35	17	1.9	1.3	190	107	83	124	8.1	16.4
65†	CG	31.0	S	0	37				4.1	9.6	156	68				
56*		34.5	M	0	66						273	175	98	88		
57*		35.5	S	0	17						101	70	31	88		
58*		37.0	S	0	4						311	114	197	144		
64a†		28.0	S	0	31						128	68	60	72		

\* Perfusate—defibrinated blood } recipient dog blood made anticoagulable by use of heparin (see text).  
 † Perfusate—heparinized blood }  
 Other symbols as in table 1.

important in determining the time of onset of failure and of survival of the heart. To these, and to experimental manipulations, must be attributed the wide variation in the time periods found.

These experiments offered no evidence that the addition of calcium gluconate with or without glucose was effective in prolonging the duration of the experi-

TABLE 3

Statistical significance of linear correlation of certain data in tables 1 and 2

x	y	ISOLATED HEART PREPARATION					HEART-LUNG PREPARATION				
		n	r	p	b <sub>1</sub>	t <sub>b<sub>1</sub></sub>	n	r	p	b <sub>1</sub>	t <sub>b<sub>1</sub></sub>
1 LVP	RVP	39	0.1175	40-50			30	0.2450	10-20		
2 LVP	AP	39	0.0610	70-80			30	0.3930	2-5	1.127	2.259
3 LVP	PP	39	-0.0270	80-90			29	0.4480	1-2	1.010	2.608
4 LVP	PF	39	-0.0090	>90			29	0.0724	70-80		
5 LVP	AF	39	0.0470	70-80			29	0.1680	30-40		
6 *LVP	CF	39	-0.4850	0.1-1.0	-3.843	3.373	28	-0.1250	50-60		
7 *LVP	CF/HW	35	-0.0560	70-80			14	-0.2750	30-40		
8 LVP	W	39	-0.0840	60-70			28	0.1880	30-40		
9 *LVP	PF/CF	39	0.1170	40-50			28	0.2200	20-30		
10 LVP	PF/HW	35	-0.0149	>90			15	-0.3291	20-30		
11 LVP	W/HW	35	0.0407	80-90			15	-0.2118	40-50		
12 RVP	AP	39	0.2450	10-20			31	0.1710	30-40		
13 RVP	PP	39	0.0445	70-80			30	0.1310	40-50		
14 *RVP	PF	39	-0.1100	50-60			30	0.0590	70-80		
15 RVP	AF	39	-0.0017	>90			30	0.0640	70-80		
16 *RVP	CF	39	-0.2358	10-20			29	0.0310	80-90		
17 RVP	HR	39	-0.2380	10-20			29	0.1320	40-50		
18 RVP	W	39	0.0810	60-70			29	0.1150	50-60		
19 RVP	PF/HW	35	0.2499	10-20			15	-0.0910	70-80		
20 RVP	W/HW	35	0.2679	10-20			15	-0.0390	80-90		
21 AP	PP	39	0.2573	10-20			30	0.3966	2-5	0.3109	2.286
22 AP	PF	39	-0.0060	>90			30	0.3620	2-5	2.8730	2.053
23 AP	AF	39	-0.6190	<0.1	-10.984	4.797	30	0.4750	0.1-1.0	2.6144	2.862
24 *AP	CF	39	-0.1326	40-50			29	0.0800	60-70		
25 *AP	CF/HW	35	-0.0655	70-80			15	0.1096	60-70		
26 AP	W	39	0.1230	40-50			29	0.5250	0.1-1.0	0.5337	3.206
27 PP	PF	39	-0.0910	50-60			29	0.5179	0.1-1.0	5.0291	3.146
28 PP	AF	39	-0.0490	70-80			29	0.3290	5-10		
29 PP	W	39	0.0398	80-90			29	0.6420	<0.1	0.7914	4.349
30 *AF	CF	44	0.2189	10-20			33	0.4030	2-5	0.2699	2.453
31 AF	PF	44	0.8420	<0.1	1.1821	10.12	33	0.9010	<0.1	1.2706	11.57
32 AF	W	39	0.8370	<0.1	0.1138	9.322	28	0.8960	<0.1	0.0161	10.30
33 *CF	PF	44	0.7325	<0.1	1.2658	6.974	33	0.7800	<0.1	1.8013	6.512
34 *CF	W	39	0.7480	<0.1	0.1187	6.863	28	0.7110	<0.1	0.2010	5.158
35 *CF	HW	35	0.6590	<0.1	0.3006	5.033	15	0.4630	5-10	0.2578	1.885
36 *CF	HR	44	0.5787	0.1-1.0	0.1670	4.598	31	0.0270	80-90		
37 *CF	PF/HR	44	0.4665	0.1-1.0	0.00643	3.418	31	0.6340	<0.1	0.0187	4.420
38 *CF	T	43	0.3098	2-5	0.01140	2.091	33	0.1560	30-40		
39 *CF/HW	PF	35	0.6830	<0.1	211.98	5.372	15	0.5200	2-5	117.39	2.193
40 *CF/HW	W	35	0.6854	<0.1	17.846	5.408	15	0.4720	5-10		
41 *CF/HW	HR	35	0.5031	0.1-1.0	24.787	3.344	15	0.3060	20-30		
42 *CF/HW	T	34	0.1696	30-40			15	0.6160	1-2	2.0556	2.827
43 *CF/HW	HW	35	0.0927	50-60			15	0.0580	80-90		
44 *100-CF	PF	35	0.7187	<0.1	190.11	5.938	15	0.4440	5-10		
HW-AP											
45 *CF/AP	PF	39	0.7800	<0.1	126.55	7.591	29	0.5810	<0.1	107.27	3.707
46 *CF/AP	W	39	0.6120	<0.1	9.2024	4.708	28	0.4340	2-5	10.027	2.457
47 PF	W	39	0.9871	<0.1	0.0896	37.58	29	0.9550	<0.1	0.1213	16.757
48 PF	HW	35	0.4683	0.1-1.0	0.1248	3.045	15	0.5880	2-5	0.1616	2.625
49 PF	HR	44	0.5931	<0.1	0.1011	4.774	32	-0.1450	40-50		
50 PF	T	43	0.5319	<0.1	0.0108	4.023	34	-0.0950	50-60		
51 PF	PP-LVP						29	0.5184	0.1-1.0	0.0657	3.150
52 PF/HR	HW	35	0.2642	10-20			15	0.7210	0.1-1.0	14.777	3.749
53 PF/HR	PF	44	0.8560	<0.1	104.66	10.734	32	0.9060	<0.1	60.853	11.726
54 PF/HR	W	39	0.8610	<0.1	10.23	10.304	28	0.8580	<0.1	7.3728	8.508
55 W	HW	35	0.4537	0.1-1.0	1.3463	2.925	15	0.5180	5-10		
56 W	HR	39	0.5564	<0.1	1.0150	4.073	28	-0.2530	10-20		
57 W	T	38	0.4770	0.1-1.0	0.1087	3.257	29	0.0940	60-70		
58 HR	T	43	0.4306	0.1-1.0	0.0513	3.055	33	0.5220	0.1-1.0	0.0534	3.411
59 AP-RVP	AF	39	0.1275	40-50			30	0.4390	1-2	1.7101	2.583
60 AP-RVP	CF	39	0.1146	>90			29	0.0780	60-70		

\* Discussed in second paper of series (8).

Symbols as in table 1.

n = number of experiments.

r = coefficient of correlation.

p = probability for chance occurrence of this degree of correlation.

b<sub>1</sub> = slope of line of regression (rate of change) of y on x.t<sub>b<sub>1</sub></sub> = ratio of arithmetic mean over standard deviation.

ment or in delaying the onset of heart failure. Our impression from preliminary experiments and from previous studies with the perfused coronary system in fibrillating hearts was not substantiated. At present, therefore, we do not employ glucose and calcium gluconate as additions to the blood perfusate. The difference between the previous and the present experiments may be due to improved skill in making these preparations.

The data on hearts in progressive failure scattered roughly over the same range as the data on hearts not in progressive failure, indicating that other factors sufficient to mask the influence of failure were at work. For this reason the data on these hearts are not separated from the other data, but they may be identified by reference to tables 1 and 2.

*The Significance of the Various Measurements and the Values Obtained.* *a. Venous pressures.* In the isolated heart, the pressure in the veins entering the left auricle (LVP) varied from 2.2 to 17.1 cm. H<sub>2</sub>O, while the pressure in the veins entering the right auricle (RVP) varied more, from 0.6 to 27.0 cm. H<sub>2</sub>O (cf. cols. 10 and 11, table 1). In the heart-lung, LVP varied from 0.7 to 25.1 cm. H<sub>2</sub>O, while RVP varied somewhat more, from 1.0 to 39.7 cm. H<sub>2</sub>O (cf. cols. 10 and 11, table 2).

In the isolated heart preparation, left venous pressure is measured at a point between the blood reservoir and the left auricle. It is determined by four factors, the driving force or pressure head, the resistance offered to flow, the state of the left heart, and the degree of distention and elastic properties of this portion of the circuit. The driving force is set at the reservoir. The resistance to flow is determined by the length and net cross sectional area of the tubing and the viscosity of the blood. (Narrow cannulae and kinking must, of course, be avoided; this was usually done successfully.) The state of the left heart expresses itself as the ability of the left ventricle to expel the blood coming to it against the resistance existing in the systemic circuit. Overdistention of the left heart, leading to mitral regurgitation, permits the backward transmission of at least part of the pressure created by the left ventricle during its systole. In the control values (table 1), this last factor was apparently non-operative. The resistance in the systemic circuit in this preparation is a twofold one, *a*, that in the extracoronary circuit, chiefly that due to the artificial peripheral resistance primarily set by the experimenter, and *b*, the resistance existing in the coronary bed; the two circuits being in parallel.

In the isolated heart preparation, right venous pressure is measured in the inferior vena cava. Its level is determined, apart from the influence of the degree of distention, on the one hand by the pressure remaining in the blood after it has passed the artificial systemic resistance interposed between the aorta and inferior vena cava. On the other hand, it is determined by the resistance to movement of blood distally, and more important, by the ability of the right ventricle to expel the blood coming to it from the inferior vena cava and the coronary bed against the resistance existing in the artificial pulmonary circuit into which it empties. This resistance is primarily determined by the setting of the artificial pulmonary resistance. The influence of kinking of the inferior



vena cava cannula, and of tricuspid regurgitation are similar to those in the left side of the heart.

The factors determining left and right venous pressure in the heart-lung preparation are similar to those in the isolated heart in most respects. They differ in that: 1, there is no fixed reservoir in the circuit, the rate of blood flow being set by varying the amount of circulating blood by means of an artificial reservoir described earlier; 2, there is no artificial resistance in the pulmonary circuit, the resistance being that of the lung circuit itself.

*b. Arterial pressures.* In the isolated heart, the aortic pressure, AP, varied between 84 and 105 mm. Hg, and the pulmonary arterial pressure, PP, varied between 3 and 24 mm. Hg (cf. cols. 8 and 9, table 1). In the heart-lung preparation, AP varied between 35 and 110 mm. Hg with most of the values above 80, and PP varied between 4 and 54 mm. Hg (cf. cols. 8 and 9, table 2). The low value of 35 mm. Hg for AP was in a heart in severe progressive heart failure.

The aortic pressure in both the isolated heart and heart-lung preparations is measured between the aortic cannula and the systemic artificial resistance. At constant blood volume, aortic pressure is affected by changes in resistance in the coronary bed as well as by the changes in the artificial aortic-vena cava path; it is an index of the total systemic peripheral resistance. The variations in the net cross sectional area of the coronary bed are dependent upon the cardiac output, upon the setting of the peripheral resistance in the aortic-vena cava path and upon spontaneous variation in the tone of its vasculature; this will be discussed in greater detail in another communication (8). The variations in the cross sectional area of the aortic-vena cava bed were controlled by adjusting the artificial peripheral resistance. Constriction of the aortic cannula and kinking at the point of its insertion, constituting a "hidden" resistance load, were kept at a minimum.

The pulmonary arterial pressure in the isolated heart is measured between the main pulmonary arterial cannula and the artificial pulmonary arterial resistance. Except for cases of kinking at the point of cannula insertion and the use of a narrow cannula which were avoided for the most part, pulmonary arterial pressure is an index of the peripheral resistance against which the right ventricle empties when cardiac output is constant.

In the heart-lung, pulmonary arterial pressure is determined by the varying rates of linear flow and the net cross sectional area of the natural pulmonary vascular bed. The latter will vary with the degree of inflation and deflation in inspiration and expiration, with the occurrence of small pulmonary vascular plugging and with the occurrence of pulmonary edema, as well as with the tone of the lung vessels. Obvious pulmonary vascular plugs and edema were not encountered at the beginning of these experiments; nor were they common in their course. Constriction and kinking of the cannulae in the right and left pulmonary artery were avoided as far as possible. In short, the heart-lung differed from the isolated heart in having more uncontrolled variables determining pulmonary arterial pressure.

*c. Pulmonary flow.* Except during periods of change, the pulmonary flow,

the measure of the output of the right heart, must be equal to the output of the left heart, that is, to the sum of the coronary flow and the flow in the aorta beyond the opening of the coronary arteries. Pulmonary flow in the isolated heart is determined, therefore, not only by the height at which the reservoir is placed and the diameter of the tube emerging from it, but also by the ability of the ventricles to pump blood against their respective peripheral resistances. As shown in column 12, table 1, the pulmonary flow, PF, varied from 98 to 750 cc./min.

The situation is analogous in the heart-lung preparation except that the pulmonary peripheral resistance is not set but is a variable depending on the conditions in the lung circuit, and the blood flow is not set by a reservoir but by adding or subtracting blood from the circuit. Pulmonary flow, as shown in column 12, table 2, varied from 98 to 547 cc./min.

*d. Aortic flow.* In both the isolated heart and heart-lung, the aortic flow, AF, is measured below the mouths of the coronary arteries; *it is thus only part of the total output of the left ventricle.* It will, of necessity, be the difference between the total output indicated by pulmonary flow and the coronary flow, except during the short intervals when conditions are altered.

The range of aortic flow in both isolated heart and heart-lung was wide; in the former (column 13, table 1) it varied from 33 to 366 cc./min., in the latter (column 13, table 2) it varied from 44 to 357 cc./min.

*e. Cardiac work.* As explained in our previous communications (2-5) the work of the heart is calculated from the cardiac output (pulmonary flow), multiplied by the sum of aortic and pulmonary arterial pressures and by a suitable constant to convert cc./min. and mm. Hg to kgM./hr. Only the part of the external work of the heart which imparts pressure to the blood leaving the right and left ventricles is considered; the kinetic energy of the blood is disregarded, since it is negligible in the range of output studied (5). The calculation uses mean values of pressure and is not as accurate as would be the case if the values were integrated (10). Since work is determined by the variable pulmonary flow and the relatively constant aortic and pulmonary arterial pressure, it would be expected that cardiac work would show relationships similar to those of pulmonary flow. This was actually the case. Cardiac work, *w*, varied from 8.3 to 66.0 kgM./hr. in the isolated heart (column 16, table 1) and from 7.9 to 62.8 kgM./hr. in the heart lung (column 16, table 2).

*f. Heart rate and blood temperature.* The heart rate in these preparations is not set by an artificial pacemaker but is determined by the spontaneous activity of an auricular pacemaker, presumably the sinus node. The rate would depend on the state of the node, and the state and temperature of the blood. In most instances the heart was regular; even in the heart-lung no arrhythmias related to respiration were noted, confirming the view that the respiratory sinus arrhythmia seen in the normal animal is neurogenic in origin. The few heart irregularities seen early in the course of the experiments were interpreted as being due to premature systoles.

The heart rate, HR, varied from 50 to 148 beats/min. in the isolated heart and

from 60 to 144 beats/min. in the heart-lung (cf. column 15, tables 1 and 2). The temperature range encountered in these experiments was 29.0 to 37.0°C in the isolated heart and 28.0 to 38.0°C in the heart-lung (cf. column 3, tables 1 and 2).

*Analysis of Graphs Involving Left Venous Pressure.* The graphs relating left venous pressure to coronary flow will be discussed in another communication (8). Of the other graphs only 3 in the heart-lung preparation showed any sort of linear correlation; none in the isolated heart preparation did (cf. table 3). The relationship between PF and PP-LVP will be considered under pulmonary flow.

In the heart-lung preparation, left venous pressure showed a statistically significant linear correlation with aortic pressure and pulmonary arterial pressure (lines 2 and 3, table 3). The statistically significant correlation of left venous pressure with aortic and pulmonary arterial pressures indicates how closely linked these pressures are in the heart-lung preparation. The lack of correlation between pulmonary arterial pressure and left venous pressure in the isolated heart preparation is due to the fact that in this preparation the interposition of the adjustable pulmonary reservoir makes the two variables independent. In the heart-lung, pulmonary arterial pressure is not artificially set, and left venous pressure equals pulmonary arterial pressure minus the pressure drop across the natural peripheral resistance of the lung blood vessels. The fact that the two show a linear relationship suggests the absence of wide variation in intrapulmonary vascular resistance. Similar considerations apply to the intact circulation; namely, that the pulmonary arterial and pulmonary venous pressures would be expected to vary in parallel fashion except when the intrapulmonary vascular resistance is altered.

The linear relation between left venous pressure and aortic pressure in the heart-lung preparation was not seen in the isolated heart preparation apparently because the presence of the adjustable pulmonary reservoir makes the two variables independent.

The lack of correlation of left venous pressure with pulmonary flow in both preparations appears to depend on the fact that the total resistance of the systemic circuit decreases as the cardiac output increases, probably due to the concomitant enlargement of the coronary bed. Since cardiac work, as mentioned earlier, is closely correlated with pulmonary flow it is not surprising that left venous pressure and cardiac work reveal no linear correlation.

*Analysis of Graphs Involving Right Venous Pressure.* The graphs relating right venous pressure to coronary flow will be discussed in another communication (8); those relating it to left venous pressure have been discussed above. In the other graphs no linear correlation was revealed (table 3) except in the relationship between AP-RVP and AF in the heart-lung preparation. This latter will be discussed under aortic flow.

*Analysis of Graphs Involving Aortic Pressure.* The graphs relating aortic pressure to coronary flow will be discussed in another communication (8), those relating to left and right venous pressure have been considered above. In the other graphs only the one relating aortic pressure to aortic flow showed a sta-

tistically significant linear correlation in the isolated heart (line 23, table 3) while in the heart-lung, aortic pressure showed a linear correlation with aortic flow, pulmonary flow, work and pulmonary arterial pressure (lines 21, 22, 26 and 23, table 3). In addition a linear correlation was found between AP-RVP and AF in the heart-lung; this will be discussed under aortic flow.

Again the statistically significant linear relationships between aortic pressure and these flows and pressures indicate how closely linked these pressures and flows are in the heart-lung preparation. The presence of the adjustable reservoir and artificial "lung" circuit in the isolated heart preparation makes these pressures and the pulmonary flow more independent variables.

The relation of aortic flow to aortic pressure is exactly opposite in the heart-lung and isolated heart. In the heart-lung, aortic pressure rises as aortic flow increases while in the isolated heart the aortic pressure decreases with rising aortic flow. When the influence of cardiac output (pulmonary flow) affects aortic pressure, as in the case of the heart-lung, a similar relation would be expected to exist, as found between aortic pressure and pulmonary flow, since aortic flow will vary with pulmonary flow. But when the influence of cardiac output (pulmonary flow) does not exert a statistically demonstrable effect, as in the isolated heart, then the effect of peripheral resistance will dominate. This implies that greater resistance in the artificial resistance in the extracoronary circuit will cause a decrease in aortic flow and simultaneously tend to increase the aortic pressure, as was found.

*Analysis of Graphs Involving Pulmonary Arterial Pressure.* The graphs relating pulmonary arterial pressure to coronary flow will be discussed in another communication (8), those relating it to aortic pressure and to left and right venous pressure have been considered above. In the other graphs, the only statistically significant linear correlations were found in the heart-lung preparation, in which pulmonary arterial pressure showed statistically good linear relations with pulmonary flow and with work (lines 27 and 29, table 3) and a statistically doubtful linear relation with aortic flow (line 28, table 3). The relation between PP-LVP and PF will be discussed under pulmonary flow.

In the heart-lung, pulmonary arterial pressure, as expected, is a dependent variable of pulmonary flow. In the isolated heart, the independent setting of the artificial pulmonary resistance vitiates this relationship. Since aortic flow is a function of pulmonary flow in the heart-lung it might be expected to show some correlation with pulmonary pressure. This illustrates the more intimate interrelationship of flows and pressures in the heart-lung preparation compared to the isolated heart preparation and this close interrelationship should hold in the intact circulation except insofar as it may be influenced by neurogenic or other factors.

*Analysis of Graphs Involving Pulmonary Flow.* The graphs relating pulmonary flow to coronary flow will be discussed in another communication (8), those relating it to the left and right venous pressures and to the aortic and pulmonary arterial pressures have been considered above.

Aortic flow in both isolated heart and heart-lung preparations showed a

statistically good linear relation with pulmonary flow (line 31, table 3). A good linear correlation also was found to exist between stroke volume and pulmonary flow (line 53, table 3) indicating, as expected, that in these preparations cardiac output variations were due principally to changes in stroke volume. However, a good linear correlation was also found to exist between pulmonary flow and heart rate in the isolated heart (line 49, table 3) indicating that heart rate played a rôle in determining cardiac output; no such correlation was found in the heart-lung preparation. A similar discrepancy in the relationship of pulmonary flow and blood temperature was found; in the isolated heart the linear correlation was statistically good, in the heart-lung it was not (line 50, table 3). A statistically good linear relation was found to exist between pulmonary flow and heart weight in both preparations (line 48, table 3) and between stroke volume and heart weight only in the heart-lung preparation (line 52, table 3), indicating that the large hearts have larger cardiac outputs and tend to have larger stroke outputs than smaller hearts.

In the heart-lung,  $\frac{PP-LVP}{PF}$  shows a positive linear correlation as expected (line 51, table 3) since the numerator is the pressure drop from the arterial to venous side of the lung circuit and this drop, assuming a fairly constant cross section in the pulmonary bed, should be a direct function of the linear velocity of flow in the lung vessels.

*Analysis of Graphs Involving Aortic Flow.* The graphs relating aortic flow to coronary flow will be discussed in another communication (8), those relating it to the venous pressures, arterial pressures and pulmonary flow have been discussed above.

Aortic flow showed a good statistically linear correlation with cardiac work (line 32, table 3) as was expected because of its close relationship to pulmonary flow. Only in the heart-lung preparation was a statistically good correlation found between aortic flow and the pressure drop between the aorta and inferior vena cava (line 59, table 3). Apparently other factors prevent this relationship from being demonstrable in the isolated heart.

*Analysis of Graphs Involving Cardiac Work.* For the reasons discussed above in explaining the calculation of work, the graphs involving work were similar to corresponding ones involving pulmonary flow, the relation between pulmonary flow and work being statistically good (line 47, table 3). As has been stated, of the factors entering into the calculation of work only pulmonary flow was varied widely. Hence it is to be expected that work and pulmonary flow correlate with the same variables.

*Analysis of Graphs Involving Heart Rate.* The graphs involving heart rate and pulmonary flow have already been discussed. Those relating it to coronary flow will be discussed in another communication (8).

As expected a positive correlation is seen between heart rate and temperature in both the isolated heart and heart-lung preparations (line 58, table 3).

While no graphs were made, a glance at columns 15 and 17 of tables 1 and 2 shows that the  $O_2$  level in the arterial blood had no apparent influence on the

heart rate. Coronary flow had no influence on heart rate (cf columns 14 and 15, tables 1 and 2). It is further apparent that the heart rate was not influenced by the product of arterial  $O_2$  content and coronary flow. Apparently the  $O_2$  level in these experiments was above the critical value which might influence heart rate.

#### SUMMARY AND CONCLUSIONS

Two preparations, an isolated heart and a closed circuit heart-lung, are described in which the dynamics of the circulation could be analyzed under controlled conditions. The main differences between the two preparations are 1, that in the isolated heart preparation the circuit is interrupted between the pulmonary artery and left auricle by an artificial "lung" and a pump for returning blood to a reservoir, while in the heart-lung preparation the lungs are left in situ (the artificial "lung," pump, artificial pulmonary peripheral resistance and reservoir being omitted), and 2, that artificial control is obtained in the isolated heart preparation by varying the artificial peripheral resistance placed in both the pulmonary and systemic circuits and/or the reservoir height, while in the heart-lung preparation artificial control is obtained by varying the artificial peripheral resistance placed only in the systemic circuit and/or the amount of blood in circulation.

A total of 79 experiments was analyzed, 44 isolated heart and 35 heart-lung preparations. Graphs of various measurements were made of all these experiments using the initial control levels. These were subjected to statistical analysis in order to gain information about the circulatory dynamics in these preparations and to further knowledge of the cardiodynamics in the intact circulation. Considerations of the control of coronary flow are deferred to a later report (8).

In general the experiments were of longer duration, progressive heart failure was longer delayed and less brusque in its development in the heart-lung than the isolated heart preparations. Among the heart-lung preparations those in which heparinized blood was used survived longer than those with defibrinated blood. The presence of progressive heart failure in some of these preparations when the initial readings were made did not affect the graphs here analyzed in any significant manner.

The significance of the various findings on the interrelation of the variables analyzed is discussed briefly.

The outstanding fact observed was the greater degree of interdependence of the various pressures and flows in the heart-lung preparation compared to the isolated heart. This is attributed to the fact that in the heart-lung preparation the main change made is in the amount of circulating blood which tends to affect all the pressures and cardiac output at the same time and in the same direction. The greater freedom of experimental adjustment in the isolated heart nullified this to a large extent. In the intact circulation, it would appear that the changes would be interrelated somewhat as in the heart-lung preparation insofar as the adjustments are due to alterations in circulating blood volume.

Change in circulating blood volume is one of the most important, but not the only means, of adjusting the dynamics of circulation in the intact animal. However, the operation of compensatory mechanisms in the intact animal, chiefly of neurogenic origin, would modify the interdependence found in the isolated heart-lung preparation.

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# THE CONTROL OF THE CORONARY FLOW IN THE DENERVATED ISOLATED HEART AND HEART-LUNG PREPARATION OF THE DOG<sup>1</sup>

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The control of the coronary circulation has been extensively investigated but agreement has not yet been attained on many aspects (1). Attention has been devoted to this subject in this laboratory for some time and our previous results have been recently summarized (2), (3). Our more recent experiences with the dynamics of the isolated heart and heart-lung preparation have afforded the opportunity of studying the changes in coronary flow under more nearly natural conditions than in the preceding types of experiments and of relating these changes to alterations in other variables.

The material of our study is incorporated in a number of graphs plotting one or more of the variables against the coronary flow at the start of the control periods of the 79 experiments used in our previous report (4). The facts established by the analysis of these graphs are supplemented by data obtained in the course of individual experiments following changes of various factors controlling the dynamics of the preparation. In our previous communication (4) we have given the details of the nature and method of establishing the preparations and the methods of measuring the various variables, and have analyzed what they represent; this report (4) should be consulted in order to facilitate comprehension of the discussion in the present report.

*Analysis of Control Values at the Start of the 79 Experiments.* The scatter graphs of the data obtained at the start of the control period of the 79 isolated heart and heart-lung preparations (shown in tables 1 and 2 of a previous communication (4)) were subjected to statistical analysis to determine whether significant linear correlations were present. The statistical data so obtained are shown in table 3 of our previous communication (4). All surviving preparations are included in this analysis. In view of the expected variations from heart to heart it appears all the more significant that the control levels of coronary flow showed certain trends and correlations.

*a. Control values and significance of coronary flow measurements.* The control values of coronary flow at the start of the experiments ranged from 39 to 392 cc./min. in the isolated heart preparations and from 6 to 197 cc./min. in the heart-lung preparations (cf. col. 14, tables 1 and 2, of previous report (4)). This was equivalent to a coronary flow of from 0.34 to 2.06 cc./gram heart/min.

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in the isolated heart and of from 0.05 to 1.67 cc./gram heart/min. in the heart-lung. Coronary flow is determined by subtracting the aortic flow, AF, measured beyond the mouths of the coronary arteries from pulmonary flow, PF, which measures cardiac output. The values of coronary flow so obtained presuppose that the outputs of the two sides of the heart are equal and this is true dynamically when conditions are stabilized. While conditions are changing it is quite possible that the two chambers do not pump the same quantity of blood. At such times the difference between pulmonary and aortic flow measures the disparity in cardiac output of the left and right heart as well as coronary flow. The error in measuring coronary flow is slight, however, except when conditions are changing rapidly. Consequently in this analysis we have used values for coronary flow only when conditions were stabilized or changing slowly.

b. *Coronary flow and heart weight.* Coronary flow was found to be linearly related to heart weight (line 35, table 3, previous report (4)) but the rate of flow per gram of heart mass does not differ in the large and small hearts (line 43, table 3, previous report (4)). Apparently the size of the coronary bed varies more or less in proportion with the mass of the heart. Because of the dependence of coronary flow on heart weight not only the total coronary flow but also coronary flow per gram of heart weight was plotted against the other variables.

c. *Coronary flow and the venous pressure levels.* No linear correlation was found to exist between the right venous pressure and coronary flow and none was found between left venous pressure and rate of coronary flow per gram of heart mass (lines 7 and 16, table 3, previous report (4)). However, a statistically good inverse correlation was found to exist between left venous pressure and total coronary flow in the isolated heart but not in the heart-lung preparation (line 6, table 3, previous report (4)). The biological significance of this correlation in the isolated heart is not clear to us, and we consider it probably fortuitous.

d. *Coronary flow and the aortic pressure level.* Surprisingly no linear correlation is found when aortic pressure is plotted against total coronary flow and against coronary flow per gram heart weight (figs. 1 and 2; lines 24 and 25, table 3, previous report (4)). These graphs demonstrate clearly that in the isolated heart and heart-lung preparation the height of the aortic pressure is not the only important factor influencing coronary flow. The resistance in the coronary bed undoubtedly is variable from heart to heart permitting larger or smaller volume flows under the same driving force.

e. *Coronary flow and the cardiac output and cardiac work.* A linear correlation is seen between coronary flow and cardiac output (pulmonary flow) (fig. 3 and line 33, table 3, previous report (4)) and between cardiac output and coronary flow per gram of heart muscle (fig. 4 and line 39, table 3, previous report (4)). Similar linear correlations are found when the cardiac output is related to the ratio of coronary flow/aortic pressure (fig. 5 and line 45, table 3, previous report (4)) and to the ratio of rate of coronary flow per gram of heart muscle/aortic pressure (line 44, table 3, previous report (4)). The ratio coronary flow/aortic pressure varies inversely with the coronary bed resistance encountered in each preparation and is therefore an indication of the latter. Apparently coronary bed resistance varied inversely with cardiac output.

As might be expected the coronary flow correlates with work in the same manner as with cardiac output (lines 34, 40 and 46, table 3, previous report (4)).

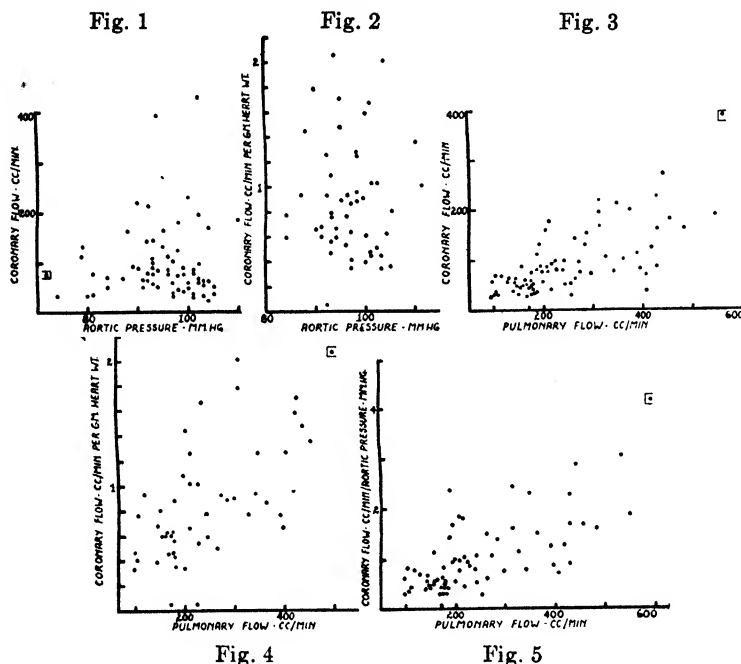


Fig. 1. Graph relating coronary flow to aortic pressure in the isolated heart and heart-lung preparations, using the control values at the start of the experiments.  $n = 29$  in heart-lung, 39 in isolated heart;  $r = 0.0800$  in heart-lung,  $-0.1326$  in isolated heart;  $p = 60-70$  in heart-lung, 40-50 in isolated heart. The value bracketed at lower left of figure is 35 mm. Hg. Discussed in text.

Fig. 2. Graph relating coronary flow per gram heart weight to aortic pressure in the isolated heart and heart-lung preparations, using the control values at the start of the experiments.  $n = 15$  in heart-lung, 35 in isolated heart;  $r = 0.1096$  in heart-lung,  $-0.0655$  in isolated heart;  $p = 60-70$  in heart-lung, 70-80 in isolated heart. Discussed in text.

Fig. 3. Graph relating coronary flow to pulmonary flow in the isolated heart and heart-lung preparations, using the control values at the start of the experiments.  $n = 33$  in heart-lung, 44 in isolated heart;  $r = 0.7600$  in heart-lung,  $0.7325$  in isolated heart;  $p = <0.1$  in both preparations. The value bracketed at upper right of this figure and in figures 4 and 5 is 750 cc./min. Discussed in text.

Fig. 4. Graph relating coronary flow per gram heart weight to pulmonary flow in the isolated heart and heart-lung preparations, using the control values at the start of the experiments.  $n = 15$  in heart-lung, 35 in isolated heart;  $r = 0.5200$  in heart-lung,  $0.6830$  in isolated heart;  $p = 2-5$  in heart-lung,  $<0.1$  in isolated heart. Discussed in text.

Fig. 5. Graph relating the ratio  $\frac{\text{coronary flow}}{\text{aortic pressure}}$  to pulmonary flow in the isolated heart and heart-lung preparations, using the control values at the start of the experiments.  $n = 29$  in heart-lung, 39 in isolated heart;  $r = 0.5810$  in heart-lung,  $0.7800$  in isolated heart;  $p = <0.1$  in both preparations. Discussed in text.

Since the cardiac work is proportional to pulmonary flow multiplied by the sum of aortic and pulmonary arterial pressures and since the pressures were kept constant within fairly narrow limits at the start of the experiments (cf. 4), it

follows that work of the heart should show the same relationships as shown for cardiac output.

Coronary flow is a function of stroke volume as well as of minute cardiac output; a linear correlation is seen between the two (line 37, table 3, previous report (4)). This is due to the fact that in our experiments variations in cardiac output occurred mainly as a result of alterations in stroke volume rather than in heart rate.

The dependence of coronary flow on cardiac output and its lack of dependence on aortic pressure are important findings for the initial state of the preparations.

f. *Coronary flow and aortic flow.* Although aortic flow and coronary flow correlate with cardiac output no correlation is seen when they are plotted against each other in the isolated heart (line 30, table 3, previous report (4)). It is apparent from this lack of correlation that the partition of cardiac output between the coronary and aortic-vena cava circuits is variable in our preparations. This partition ratio furthermore is found to be independent of cardiac output. Our findings emphasize the unreliability of considering aortic flow below the mouths of the coronary arteries as an index either of coronary flow or total cardiac output.

In the heart-lung, a significant correlation was found between aortic and coronary flows.

g. *Coronary flow and arterial oxygen level.* As the data (cols. 7, 14 and 17, tables 1 and 2) of our previous report (4) show, there is no correlation between coronary flow or coronary flow per gram of heart muscle and arterial  $O_2$  content, when the control periods of various experiments are compared. Apparently, the oxygen content in the arterial blood in these experiments was well above the critical level at which oxygen content affects coronary caliber (6).

\* h. *Coronary flow and blood temperature and heart rate.* Graphs relating coronary flow to heart rate and temperature, lines 36, 38, 41 and 42, table 3, previous report (4), show that for the range of temperature investigated there is a linear correlation such that the coronary flow per gram of heart muscle tends to be high with higher temperatures in the heart-lung and with higher heart rates in the isolated heart. The correlations of coronary flow per gram of heart muscle with temperature and with heart rate may be independent of each other or interdependent since heart rate has been found to be a definite function of temperature. The answer to this question will be deferred until data in single experiments are presented below.

i. *Coronary flow and the pressure drop between the aorta and right auricle.* The graph  $\frac{AP-RVP}{CF}$ , in which the numerator is the pressure drop in the coronary

bed and the denominator is the coronary flow, failed to reveal any linear correlation (line 60, table 3, previous report (4)). This is in contrast to the linear

correlation seen in the heart-lung in the graph  $\frac{AP-RVP}{AF}$  (4), indicating that the

great variation in cross section area of the coronary bed from preparation to preparation prevents the pressure drop from giving any indication of the magnitude of coronary flow. It reveals one of the defects of indirect measures of coronary flow employed in the past which depend on changes in the pressure drop in the coronary bed.

*Results Obtained During the Course of Individual Experiments.* a. *Spontaneous variations in coronary flow during the course of an experiment.* It was found that in many preparations there was a spontaneous increase in coronary flow in the course of the experiment. This spontaneous increase occurred independent of any increase in cardiac output. It had been seen previously in experiments in which the ventricles were deliberately kept fibrillating (7). Heart failure was not essential for its occurrence; it often occurred in the absence of heart failure (fig. 6 and fig. 7, after 48 min., also fig. 3 of another report (5), figs. 2 and 3 of another report (11) and fig. 3 of another report (16)). The increase in coronary flow must have been due to some change in the perfusing blood or within the heart itself leading to vasodilatation of a humoral nature. In some cases, but not in all, the reduction in coronary resistance was enough to reduce the *total* peripheral resistance in the systemic bed and therefore to become evident as a fall in aortic pressure (fig. 6). In short, a spontaneous rise of coronary flow was seen with constant cardiac output with or without a fall in aortic pressure. This observation has led us to consider that the ratio aortic pressure/coronary flow is a better index of coronary bed resistance than coronary flow alone.

Spontaneous coronary dilatation led to a redistribution of blood flow, a greater fraction of the cardiac output being diverted through the coronary circuit and a smaller fraction running off through the parallel extracoronary systemic circuit, the aortic-vena cava path. This demonstrates again the error in assuming 1, that coronary flow remains constant in the course of an experiment, and 2, that aortic flow is a constant fraction of total cardiac output, and therefore is a reliable index of the latter, as an experiment progresses. Both these assumptions are inherent in some published results which have utilized aortic flow as an index of cardiac output. The unreliability of determinations of cardiac work and mechanical efficiency based on these assumptions is obvious.

In many experiments, spontaneous coronary dilatation led us to increase the systemic peripheral resistance in order to maintain the aortic pressure constant (fig. 7, after 43 min., and fig. 3 of another report (5)). This maneuver of course made the absolute and relative increase in coronary flow even greater than it would have been due to spontaneous coronary dilatation alone.

In the presence of spontaneous coronary dilatation it was difficult to judge whether or not a deliberate alteration in a factor controlling the dynamics of the preparation had an effect on coronary flow. Our criterion for such an effect was a sharp change in the slope of the coronary flow curve plotted against time and a return to approximately the pre-existing slope when the previous conditions were restored. Fortunately there was a sufficient number of experiments in which this spontaneous coronary flow change was absent for a long time, or did not occur at all, thus enabling us to make correlations more certain.

b. *Effect on coronary flow of change in cardiac output in a single experiment.* If the systemic peripheral resistance is left unaltered, a change in cardiac output (pulmonary flow) causes a change in the coronary flow in the same direction. In some instances (fig. 7, 1st 43 min., fig. 2 of another report (17), fig. 4 of another report (11) and fig. 2 of another report (5)) the aortic pressure remains unaltered; in others, it changes in the same direction (fig. 8). Even when the peripheral

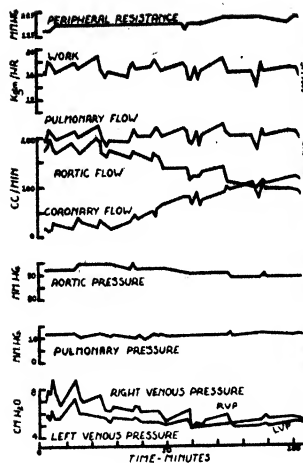


Fig. 6

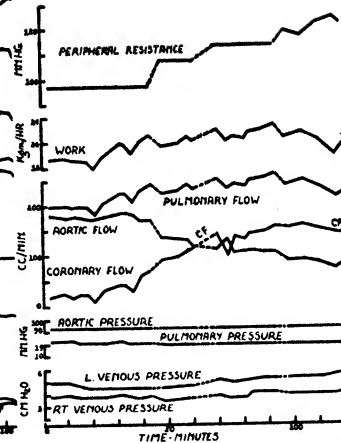


Fig. 7

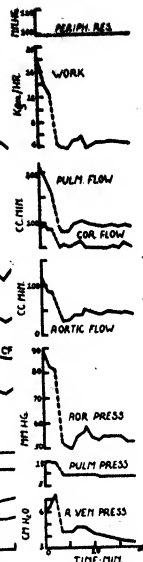


Fig. 8

Fig. 6. Chart showing pertinent data of a portion of a heart-lung experiment prior to the onset of progressive heart failure. Spontaneous coronary dilatation is manifest by an increasing level of coronary flow. This dilatation led to a fall in aortic pressure. The general levels of cardiac output (pulmonary flow) and of the peripheral systemic resistance were constant. Evidence of improvement of the heart is found in this experiment in the early drop in the venous pressure levels while the general level of cardiac output is unchanged. The fluctuations in cardiac output are mirrored in the curve of cardiac work, and for the most part also in coronary flow and in the venous pressures, especially the right. This experiment illustrates that aortic flow is not an index of cardiac output (pulmonary flow). *RVP* and *LVP* represent the right and left venous pressures. *KgM.* is kilogram-meters in this and other figures. Discussed further in text of this and a subsequent report.

Fig. 7. Chart showing pertinent data of a portion of a heart-lung experiment. During the latter part of the chart there is evidence of mild progressive heart failure in the slight elevation of the venous pressures in the absence of change in aortic and pulmonary arterial pressure and in the absence of a rise in cardiac output. In the first 43 minutes coronary flow changes parallel the increase in pulmonary flow after which the coronary flow increase was due to spontaneous coronary dilatation, the general pulmonary flow level remaining constant. This was augmented by the progressive increase in peripheral systemic resistance necessitated in order to keep the aortic pressure from falling. The last increase in peripheral resistance did not increase coronary flow apparently because the cardiac output (pulmonary flow) was lowered at the same time thereby neutralizing the effect of the peripheral resistance increase. The first part of the experiment before the peripheral resistance is altered is an example of a change in coronary flow accompanying a change in cardiac output without any measurable alteration in aortic pressure being present. The experiment is another illustration of the inability of aortic flow to depict the changes in cardiac output (pulmonary flow). *L.* is left, *Rt.* is right, *CF* is coronary flow. Dotted lines in this and later figures are used to depict periods when no readings were taken. Discussed further in text of this and subsequent reports.

Fig. 8. Chart showing pertinent data of a portion of a heart-lung experiment prior to the onset of progressive heart failure. Left venous pressure was not recorded. It shows the decrease in coronary flow accompanying a decrease in pulmonary flow. In this experiment no attempt was made to maintain the aortic pressure constant by adjustment of the peripheral systemic resistance. Hence the aortic pressure fell and with it the pulmonary arterial and the right venous pressures. At the same time aortic flow and cardiac work declined. *Periph. Res.* is peripheral resistance, *Pulm.* is pulmonary, *Cor.* is coronary, *Aor.* is aortic, *Press.* is pressure, *R. Ven.* is right venous. Discussed further in text of this and a subsequent report.

resistance is changed in the opposite direction to keep aortic pressure constant, coronary flow may still follow cardiac output (cf. fig. 9, 1st 54 min.) although the change is less marked. From experiments in which the cardiac output is deliberately altered two facts stand out:

1. Changes in cardiac output do not always cause changes in aortic pressure.
2. Changes in cardiac output lead to changes in coronary flow in the same direction even when the aortic pressure is unaltered.

The explanation for the first finding is that the aortic pressure level is an index of total peripheral resistance, that is, the resistance in the two parallel systemic circuits, the coronary and the extracoronary systemic circuits (cf. fig. 12). Increase in cardiac output tends to increase the content of these beds, distending them, and thereby automatically reducing their resistance to flow. The relation, however, is not linear. Aortic pressure, AP, equals the cardiac output, CO, times total peripheral resistance, TPR. It is clear that AP can be altered by changes in CO or TPR. For a rigid system TPR is constant and AP varies only with CO. This obviously does not hold in the vascular bed where the tubes are elastic. Changes in pressure within the vessel walls will cause distention and hence change in the resistance to flow. From Poiseuille's law we know that the resistance to flow varies inversely as the fourth power of the radius or since radius is proportional to pressure, inversely as the fourth power of the pressure. But AP equals CO times TPR, and if TPR is reduced by increased CO, AP will increase less than if TPR were constant. Thus, it is possible in elastic tubes (or vessels) for cardiac output to increase greatly with very small, even imperceptible, change in pressure. The degree to which this factor affects pressure-flow relationships (in this case AP-CO relationship) will depend upon the elasticity of the vessels. This is unknown and varies greatly from animal to animal and when conditions are altered.<sup>4</sup>

<sup>4</sup> A more precise definition of these facts follows:

Consider a stretch of elastic vessel of length  $l$  and average radius  $r$ . (The radius will be larger at the entrance to the segment than at its end, since the pressure is greater at the entrance.) Let  $p$  = pressure at entrance; pressure at end = 0. Average pressure =  $P = \frac{p}{2}$ . Let  $V$  = volume rate of flow.

If vessel obeys Hooke's Law (with respect to changes in radius caused by pressure changes), then

$$r = r_0 + CP$$

where  $C$  = a constant and  $r_0$  = unstretched radius (radius at 0 pressure).

$$\text{Now } p = \frac{K^4 \eta l}{r^4} V,$$

$$\text{and } 2P = \frac{K^4 \eta l}{(r_0 + CP)^4} V.$$

Upon expanding and multiplying by  $(r_0 + CP)^4$ , and letting  $K = K^4 \eta l$  we have:

$$aP + bP^2 + cP^3 + dP^4 + eP^5 = KV$$

$$\begin{array}{ll} \text{where: } a = 2r_0^4 & c = 12r_0^3 C^2 \\ b = 8r_0^3 C & d = 8r_0^2 C^3 \quad e = 2C^4. \end{array}$$

When  $C = 0$  (rigid tube), then  $2r_0^4 P = KV$ .

When  $C$  is small (slightly distensible tube) and  $r_0$  is large, the  $P^5$  term is negligible, but terms in lower powers become significant.

When  $C$  is large, all terms beyond the first become significant.

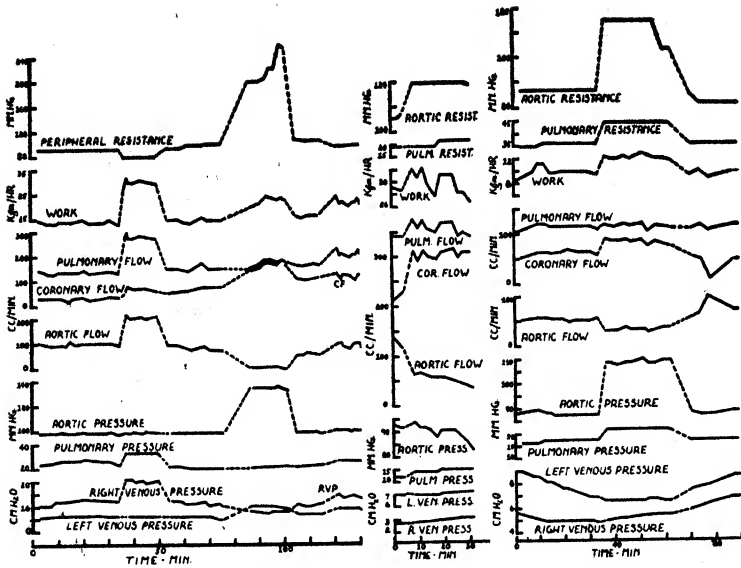


Fig. 9

Fig. 10

Fig. 11

Fig. 9. Chart showing pertinent data of a portion of a heart-lung experiment without progressive heart failure, in which cardiac output (pulmonary flow) and peripheral systemic resistance were deliberately altered. There was evidence of a slight spontaneous coronary dilatation in the general rise of the coronary flow curve, but not sufficient to obscure the induced changes. Between 35 and 54 min. pulmonary flow was temporarily increased and at the same time the peripheral resistance was decreased in order to keep the aortic pressure constant. The result was a slight but definite increase in coronary flow, despite the tendency of the peripheral resistance change to decrease coronary flow. At the same time, right venous and pulmonary arterial pressure rose but left venous pressure, following the aortic pressure, remained constant. The rise of the former pressures is an additive effect of the effects of the increased cardiac output (pulmonary flow) and the decrease in systemic peripheral resistance, the former being the more dominant. Between 76 and 105 min. the peripheral systemic resistance was temporarily increased and the pulmonary flow little altered. This resulted in redistribution of the cardiac output, more of it being diverted through the coronaries and less appearing in the extracoronary aortic flow. This illustrates again that aortic flow is an inaccurate measure of cardiac output. At the same time, aortic pressure was elevated and with it the left venous pressure. The pulmonary arterial pressure was not affected, the right venous pressure, on the other side of the peripheral resistance, fell slightly as the resistance was increased. The sharp rise in aortic pressure caused the cardiac work to increase out of proportion to the small change in pulmonary flow. The last period, in which a small increase in cardiac output was produced with a simultaneous small decrease in peripheral resistance had an effect on the other variables similar to the first period when similar but greater alterations in cardiac output and peripheral resistance were produced. RVP is right venous pressure, CF is coronary flow. Discussed further in text of this and a subsequent report.

Fig. 10. Chart showing pertinent data of a portion of an isolated heart experiment prior to and during early progressive heart failure. The latter is indicated by the fall in aortic pressure, with both the aortic peripheral resistance and pulmonary flow kept at a constant general level. The primary purpose of this figure is to show a sharp increase in coronary flow caused by a sharp rise in the aortic peripheral resistance without any accompanying elevation in aortic pressure. The small increase in pulmonary flow contributes to this effect. This experiment illustrates that the increase in coronary flow is caused by the increased

The actual level at which aortic pressure no longer follows CO varied from preparation to preparation, and as the heart failed the level of maximum aortic pressure obtainable even with increase in cardiac output was lowered (cf. 11). The former can be explained by differences in volume elasticity coefficient of the coronary bed in different preparations, the latter by the fact that the heart enlarges as it fails. Similar changes in the level at which no demonstrable aortic pressure change occurs would also result from alterations in blood viscosity and vascular muscle tone.

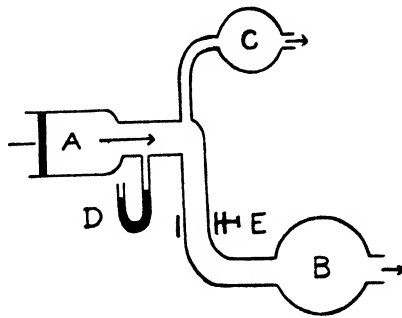


Fig. 12. Schematic diagram to represent the partition of the left heart output into two parallel circuits as exists in the isolated heart and heart-lung preparations. Piston *A* is the left ventricle pumping blood into the two parallel circuits having variable capacity by virtue of their elasticity. *C* represents the coronary bed, and *B* the extracoronary systemic circuit. *D* is a mercury manometer measuring the pressure in the common trunk, the aorta. *E* represents the adjustable artificial systemic resistance. It is obvious that increased pumping at *A* will increase the flow in *C*, and it is also obvious that increased compression at *E* will divert more blood to circuit *C*. Discussed in text.

The experimental correlation of coronary flow with cardiac output is applicable to the intact circulation in animals and man. Whatever the rôle of the nerves may be in the innervated heart, these experiments clearly show that a self-

peripheral resistance in the extracoronary circuit per se and not necessarily through the intermediary of an increase in aortic pressure. The effect of increasing pulmonary peripheral resistance on pulmonary arterial pressure is also seen. This experiment illustrates once again the inaccuracy of aortic flow as a measure of cardiac output. *Resist.* is resistance, *Pulm.* is pulmonary, *Cor.* is coronary, *Press.* is pressure, *L.* and *R.* are left and right, *Ven.* is venous. Discussed further in text of this and subsequent reports.

Fig. 11. Chart showing pertinent data of a portion of an isolated heart experiment in which cardiac output was kept constant and the peripheral resistance in the pulmonary and extracoronary systemic circuits was temporarily increased between 34 and 71 minutes. The increase in the extracoronary systemic resistance led to a redistribution of the cardiac output, the aortic flow decreasing and the coronary flow increasing. As expected, the arterial pressures rose during this period and the work of the heart increased. The changes in venous pressure appear to be independent of the induced changes in the peripheral resistances. At constant cardiac output, the early fall in the venous pressure suggests spontaneous improvement of the heart, and the later rise in the venous pressures with cardiac output constant indicates the development of progressive heart failure. Discussed further in text of this and subsequent reports.



regulating mechanism adjusting the coronary flow to the cardiac output is present in the denervated heart. This occurs despite the tendency of the extravascular forces which are increased by increasing heart output to decrease coronary flow, when the coronary circuit is fed from a separate circuit (8)<sup>5</sup>. Apparently when the coronary circuit shares the left ventricular output with the other parts of the systemic circuit, whether this latter be the natural one, or as in these experiments, an artificial one, then some mechanism overcomes the contrary action of the extravascular forces, and coronary flow increases. This is not due to any direct or indirect nerve action since these preparations are denervated and isolated. It is possible that this may be due to an accumulation of vasodilator metabolites when the heart works harder and so augments its energy exchange; this mechanism does operate in ischemia (6) and it may operate in some instances when augmentation of cardiac output leads to relative ischemia. The phenomenon can be explained on a purely mechanical basis. Obviously, if the left ventricle pumps out more blood, this must run off through the peripheral circuit, that is, through the coronary bed and through the other systemic paths. If no active change in caliber of any of these vessels occurs the increase in flow will be shared more or less proportionally by all parts of the systemic circuit, including the coronary bed. In short, on a purely mechanical basis of a simple sort it would follow that the coronary flow would be a function of the cardiac minute output. Other influences act simply to modify this fundamental correlation. This mechanical dependence of coronary flow on cardiac output is an important *law of the heart* which apparently hitherto has been overlooked. Such a dependence of coronary flow on cardiac output has been reported by Rein (9) in the intact animal but was ascribed by him to neurogenic and humoral factors. Our results show that these factors are not necessary, the correlation being explainable entirely on a mechanical basis.

Contrary results reported in the literature are due, we believe, to several factors: 1, studying the coronary circuit perfused from a separate circuit, as in our earlier studies (8); 2, comparing coronary flow with the flow in the rest of the systemic circuit and assuming that the latter is the total cardiac output, and 3, ignoring the fact that variations in the resistance of the peripheral systemic bed outside the coronary bed alter the partition between extracoronary systemic flow and coronary flow. In many experiments on cardiac output, endeavors are made to keep the aortic pressure artificially constant. When the cardiac output is increased, aortic pressure tends to rise; to overcome this the peripheral resistance is decreased, and hence blood flow is diverted into the peripheral extracoronary circuit. For example, this will explain the relatively small increase in coronary flow observed following rises in cardiac output in figure 9, first 54 minutes, since peripheral resistance had to be decreased to keep aortic pressure constant. If the decrease in peripheral resistance is made great enough the

<sup>5</sup> However, changes in the degree of extravascular pressure created in the walls of the heart and in the pressure in the right heart cavities into which most of the coronary blood drains (10) do have an influence; obviously if the cardiac output is unchanged, increases in these two factors, as might occur in right heart failure, will tend to decrease coronary flow.

simultaneous increase of cardiac output and decrease of peripheral resistance may leave coronary flow unchanged (cf. fig. 3 of previous report (13), first 70 min.<sup>6</sup>) or even decrease it.

This adjustment of coronary flow by passive change in the caliber of the coronary bed brought about by variations in cardiac output provides an automatic adjustment of coronary flow as the heart work and energy expenditure alter. The coronary flow is kept at a minimum when the cardiac output is low and it increases when the cardiac output augments, thus tending to prevent coronary insufficiency. In the presence of coronary sclerosis with narrowing and less distensible vessels or in the presence of coronary closures it is obvious that this mechanical adjustment of coronary flow to increases in cardiac output will be lessened, permitting coronary insufficiency to occur. Coronary disease, therefore, may bring about an impairment of the normal mechanical adjustment of coronary flow to cardiac output, thus limiting the ability of the heart to cope with the changes in venous return constantly occurring during activity.

*c. Effect on coronary flow of changing the systemic peripheral resistance in a single experiment.* Peripheral resistance was altered during the course of an experiment either arbitrarily to study the effects of this step, or when necessary to keep the aortic pressure constant: 1, when spontaneous coronary dilatation occurred and 2, when cardiac output was deliberately altered. It has been pointed out above that such peripheral resistance alterations cause a redistribution of the cardiac output between the coronary and extracoronary paths. An increase in the systemic peripheral resistance increased coronary flow (fig. 9, 54 min. and later, figs. 10 and 11, and fig. 2 of another report (11) in the last 7 min.); a decrease, decreased coronary flow (fig. 9, 87 min. and later, fig. 11). In short, induced changes in the systemic peripheral resistance altered the partition of the cardiac output between aortic and coronary flow, just as did spontaneous variations in coronary circuit resistance (fig. 6, fig. 7 after 43 min.).

While alterations in the systemic circuit resistance under constant cardiac output caused similar variations in coronary flow, they did not always cause similar changes in aortic pressure. For example, increasing the resistance of the extracoronary systemic circuit led to an increase in coronary flow whether the aortic pressure rose (figs. 9 and 11) or not (fig. 10 and fig. 2, last 7 min., of another report (11)). It is thus apparent that the changes in coronary flow accompanying alterations in systemic peripheral resistance are not secondary to increases in the aortic pressure. The partition of flow between these two beds (C and B, fig. 12) is dependent on their relative resistance while the aortic pressure is dependent on the total peripheral resistance,  $R_T$ .  $R_T$  is determined by  $\frac{1}{R_T} = \frac{1}{r_c} + \frac{1}{r_s}$  where  $r_c$  is the coronary bed resistance and  $r_s$  that of the extracoronary systemic bed. It is obvious from this relationship that  $r_c$  and  $r_s$

<sup>6</sup> In this experiment there was a spontaneous coronary dilatation causing coronary flow to increase steadily. At the time pulmonary flow was increased, systemic peripheral resistance (not shown) was reduced to keep aortic pressure constant. No change in the slope of the coronary flow curve is discernible.

could change simultaneously in such directions and magnitudes as to leave  $R_T$  unchanged. Thus when  $r_c$  is raised the increased flow in the coronary circuit distends the coronary bed and automatically decreases its resistance. The presence or absence of elevation in aortic pressure will thus depend on the extent to which the coronary resistance decline neutralizes the increase in the extracoronary systemic resistance.

To repeat, it is apparent from the foregoing discussion that readjustments in the distribution of blood between the aortic-vena cava and coronary circuits are not necessarily accompanied by changes in aortic pressure and that change in aortic pressure does not by itself predicate corresponding change in coronary flow. The important co-determining factor is the degree of resistance set in the artificial systemic (aortic-vena cava) circuit. This concept cannot be stressed enough. It is the resistance in the systemic circuit which is important; the diversion of blood into the coronary circuit which results when it is increased, decreases the coronary resistance and hence lessens or prevents the rise in total peripheral resistance. In this way, aortic pressure, the measure of total peripheral resistance, rises little or not at all. This mechanism probably operates also in the intact animal. By narrowing of some of the major systemic pathways, coronary flow changes may be brought about without much change in blood pressure. The application of this principle to the partition of flow among other circuits in the systemic circulation is obvious.

The futility of judging coronary flow solely from the level of the aortic blood pressure is apparent. This is not to be taken to mean that increasing the pressure head at the mouths of the coronary arteries does not cause an increase in coronary flow, but rather that other more important factors come into play which operate 1, to lessen this effect, and 2, to reduce the pressure head on a purely mechanical basis of coronary distention.

Both Anrep and King (12) and we (8) have shown that when the coronary circulation is fed from an independent source so that the driving force is not dependent on the vigor of the heart, as is the case in the intact animal, then the coronary flow depends on the pressure level of the reservoir feeding the coronary vessels. The objection was raised (8) that in Anrep and King's experiments (12) this finding may have been due to a redistribution between the perfused and non-perfused coronary arteries. This objection does not apply to our earlier work (8) since *all* the coronary vessels were perfused by the separate reservoir. Thus, there is no doubt that in the isolated coronary circuit one of the important factors controlling flow is the pressure level in the chamber feeding these vessels. However, this does not imply that in the isolated heart or heart-lung preparation the most important factor is the aortic pressure level as Anrep (14) and, more recently, Hausner, Essex et al. (15) have stressed, to the point of minimizing the effect of cardiac output on coronary flow. Our data show that aortic pressure is far from the most important factor controlling flow in such preparations and this should apply to data in animals and man when the circulation is intact. A great deal of the earlier interpretations based on the assumption that aortic pressure is the important controlling factor, and ignoring the rôle of cardiac

output and the state of vasomotor tone in the extracoronary peripheral vascular beds, will need to be reinterpreted on the basis of the present findings.

d. *The influences altering coronary flow in the isolated heart and heart-lung preparations.* The foregoing discussion shows that an important factor determining coronary resistance is the quantity of blood which the coronary bed contains. So much attention has been paid to driving force, extravascular resistance and active changes in coronary caliber that the important effect of purely passive changes in its caliber has not been sufficiently considered.

Thus the two most important influences adjusting coronary flow in the isolated heart and heart-lung preparations and presumably in the intact circulation are mechanical factors not involving nerve reflexes or humoral mechanisms, which vary the passive distention of the coronary bed, namely, 1, the minute cardiac output, and 2, the magnitude of the resistance of the extracoronary systemic beds. Before any other particular influence on coronary flow is analyzed, the question should be answered whether or not the coronary flow change obtained is a measure of the alteration in one or both of these two factors produced by the variable under consideration. When this rule is applied the rôles of aortic pressure and cardiac extravascular compression of the coronaries in the intact circulation may be found to be of little significance by comparison.

The influence of nerve reflexes, drugs and humoral substances on coronary caliber is, of course, not denied, but in each instance a direct effect must be distinguished from the effects *secondary* to changes in cardiac output and extracoronary peripheral systemic resistance because obviously the direct effect on coronary caliber may be masked or reversed. Doubtless much of the controversy concerning the factors affecting coronary flow has arisen because cardiac output and extracoronary systemic resistance were not kept constant or their influence was not considered when they were changing.

e. *The effect on coronary flow of change of temperature and of heart rate in a single experiment.* The change in temperature during the course of an experiment was usually less than  $1^{\circ}\text{C}$ ., rarely it amounted to 2 or  $3^{\circ}\text{C}$ . No striking change in coronary flow accompanied these larger changes in temperature which could not be attributed to changes in either cardiac output or peripheral resistance of the extracoronary systemic circuit, or to spontaneous increases of coronary flow. A similar situation was found with regard to spontaneous changes in heart rate, which, in a single experiment, amounted usually to an increase or decrease of the order of 10 beats/min. Reliance, therefore, was placed on several experiments in which heart rate was deliberately changed either with a Lewis interruptor stimulating the auricles or by heating and cooling the sinus node with test tubes containing warmed and cooled water. These experiments were all done with isolated heart preparations. Fourteen such changes in rate were produced and the effects analyzed. In all instances the heart rate change was associated with changes in pulmonary flow and/or peripheral resistance of the extracoronary systemic bed and the changes in coronary flow could be readily attributed to the latter changes (cf. figs. 4, 5 and 6 of another report (5) and legends for details). Depending on circumstances, in this small series we

obtained instances in which increased heart rate was associated with 1, increased coronary flow due either to an increased cardiac output or increased resistance of the extracoronary systemic circuit (fig. 4, 1st part, of another report (5)); 2, a decreased coronary flow due either to a decreased cardiac output or decreased resistance of the extracoronary systemic resistance (fig. 5 of another report (5)) or 3, little or no change in coronary flow when cardiac output and extracoronary systemic resistance changed in opposite directions (fig. 6 of another report (5)) or were unchanged (fig. 4, 2nd part, of another report (5)).

Apparently from these results, the contradictory changes in coronary flow reported in the literature may be due to varying secondary effects following spontaneous or deliberate alterations in cardiac output and changes in systemic peripheral resistance, the latter produced to keep aortic pressure constant. Whether or not heart rate changes per se, by altering the ratio of time spent by the heart in each of its cyclic phases, cause changes in coronary flow, we cannot state from our present data. Our previous studies with the separately perfused coronary circulation (8) show that heart rate changes per se do have an effect when the coronary vessels are separately perfused.

The absence of any demonstrable *direct* correlation between heart rate and coronary flow in the isolated heart preparation when heart rate was deliberately altered leads to the conclusion that the changes of coronary flow per gram of heart weight, reported above, when comparing control periods of different preparations, are more likely due to the temperature change rather than to heart rate change unless the relationship found is coincidental. The mechanism presumably would be a humoral one due to the accumulation of vasodilator metabolic byproducts in greater quantities at the higher temperatures.

#### SUMMARY

Coronary flow was studied in isolated heart and heart-lung preparations of dogs by examination and analysis of graphs correlating one or more factors with coronary flow in the control period at the start of each of 79 experiments, and by data obtained in individual experiments following alteration of the various factors controlling the dynamics of the preparation.

In the graphs, coronary flow at the beginning of the experiments was found to vary with heart weight, but not with venous or aortic pressure levels. On the other hand, there was striking correlation of cardiac output (measured as pulmonary flow) with coronary flow, index of reciprocal of coronary resistance ( $\frac{\text{coronary flow}}{\text{aortic pressure}}$ ), coronary flow per gram heart weight and  $\frac{\text{coronary flow}}{\text{aortic pressure}}$  per gram heart weight. Correlations of work of the heart were similar to those of pulmonary flow since in our preparations the latter is the main variable in calculation of cardiac work. Coronary flow was found to be independent of aortic flow and of arterial oxygen level (of the range in our experiments) but coronary flow per gram heart weight tended to increase with increase of temperature or heart rate. The pressure drop between the aorta and right auricle in the heart-lung preparation correlated with aortic flow but not with coronary

flow, indicating great variation in cross section of the coronary bed from preparation to preparation.

Examination of the course of individual experiments explained the above correlations and lack of correlations. They showed that the two most important mechanical factors influencing coronary flow in the isolated heart and heart-lung preparations, and presumably in the intact circulation, are cardiac output and systemic peripheral resistance—apart from spontaneous changes in coronary resistance which tend to occur during the course of the experiments. Thus, both increase in minute cardiac output and increase in extracoronary vascular resistance, increase coronary flow.

These two factors act automatically, and mechanically, without involving nerve reflexes or humoral mechanisms to bring about their effect. Their importance has hitherto not been properly appreciated.

Aortic pressure, which has an important influence on coronary flow in artificial preparations in which the coronary circuit is dissociated from the systemic circuit, does not play as important a part in the intact circulation. In the intact circulation, changes in aortic pressure are usually coincidental, secondary to change in cardiac output, systemic peripheral resistance, or both. The aortic pressure change tends to be minimized as a result of lessening of the change in total peripheral resistance by the alteration in coronary resistance brought about in turn by the passive alteration in the diameter of the coronary vessels. Change in coronary flow due to the predominant influence of one or both of the two factors mentioned above may occur in the absence of, or with similar or opposite, change in aortic pressure.

The degree of cardiac extravascular coronary compression is not as marked an influence as these other two factors and its effect is obscured. Similarly the effect of cardiac output and peripheral resistance may mask or reverse the influence of nerve reflexes, drugs, and humoral substance on coronary caliber and coronary blood flow.

In experiments in which heart rate was deliberately altered there was no predictability in the change in coronary flow, since coronary flow changes occurred as the result of change in cardiac output or peripheral resistance, and no effect of heart rate per se could be distinguished.

The lack of relationship of coronary flow to aortic flow in the control periods of different preparations compared to the correlation of coronary flow with pulmonary flow, and the similar variability of aortic flow with respect to both coronary flow and pulmonary flow during the course of individual experiments, shows the fallacy in assuming, as some have done, that aortic flow below the mouths of the coronaries is an accurate index of either coronary flow, total cardiac output, cardiac work or stroke volume.

We are indebted to the members of the department for assistance in the performance of these experiments, to Mrs. B. Lendrum and J. Meyer for suggestions in preparing this report and to Mrs. J. Meyer and Mr. H. Silverstone for assistance in the statistical evaluation of the data.

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# THE DYNAMICS OF THE NON-FAILURE PERIOD OF THE ISOLATED HEART AND HEART-LUNG PREPARATION<sup>1</sup>

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The dynamic and energetic changes occurring in spontaneous heart failure were investigated in the denervated isolated heart and closed circuit heart-lung preparations described in a previous communication (1). In order to appreciate the changes attributable to heart failure, it is important to ascertain the range of variation and the interdependence of the various pressures and flows before the heart goes into failure. In this report, we will therefore consider the stability of the various pressures and flows when conditions are not deliberately altered, and the interrelation of these factors when one shows a spontaneous change or when such a change is deliberately induced during the period in which evidence of progressive heart failure is absent, or failure is developing very slowly. This information is important in determining which of the changes encountered in heart failure are attributable directly to the loss of power of the heart and which are secondary to the adjustments which this loss of power necessitates. The latter, of course, can be considered as peculiar to the failing heart only if similar changes cannot be brought about by the same adjustments in the non-failing heart. Lack of appreciation of this may lead, as it doubtless has done in the past, to an erroneous conception of the changes primarily resulting from failure of the heart.

The period of progressive heart failure in these experiments was assumed to have begun 1, when, with the cardiac output kept constant, the right and/or left venous pressures rose progressively, or 2, when, in some of the isolated heart preparations, with the venous inflow pressure head not lowered, the cardiac output decreased and/or one or both venous pressures rose and/or the aortic pressure fell. The reasons for the use of these criteria will be discussed in a later communication (2).

All preparations having a non-failing control period are included, except when there was a paucity of readings in the control period, when the control period lasted less than 15 minutes, or when marked irregularity of cardiac action was present. The control period was considered terminated when progressive heart failure set in, when drugs (aside from heparin, glucose, NaCl or Ca gluconate, initially added) were administered or when gross cardiac irregularity developed. Thirty-eight experiments with valid control periods are used in this analysis, 13 on isolated heart and 25 on heart-lung preparations. The duration of the

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control periods varied from 19 to 190 minutes. In 6 of the isolated heart preparations, the pressure head of the venous reservoir connected to the left auricle was maintained constant. In some, however, the attempt was made to adjust it in order to maintain the cardiac output constant. The aortic and pulmonary arterial pressures were kept constant in all but 2 of the preparations, as far as possible, by adjusting the artificial systemic and pulmonary resistances. In the heart-lung preparation, the aortic pressure and cardiac output were kept as constant as possible by adjusting the systemic artificial peripheral resistance and the total blood volume in the preparation.

In a few experiments, these adjustments in both the isolated heart and heart-lung did not achieve the desired constancy of pressures and cardiac output. In the course of other experiments the cardiac output and the peripheral resistances were deliberately varied. In addition, spontaneous changes were encountered. Thus, in some experiments, there was evidence of cardiac improvement, evidence of spontaneous changes in coronary flow (already discussed (3)), and, in the heart-lung preparation, evidence of change in the resistance in the lung circuit caused by the development of pulmonary thrombi, pulmonary edema or by spontaneous changes in tone of the pulmonary vasculature. Occasionally, changes in blood temperature occurred, and the heart rate altered. In several experiments changes in heart rate were deliberately induced.

When adjustments are adequate and spontaneous changes in the coronary resistance, pulmonary resistance and heart rate are absent, the non-failing heart will maintain a constant output and constant pressure levels in the veins and arteries for the duration of the experiment (fig. 1). When, however, the adjustments are inadequate, when deliberate changes are made or when spontaneous variations occur, flows and pressures change. In the present report, the attempt is made to analyze these changes. The correlation of the spontaneous or deliberate changes in one or more of the variables with the associated changes in the other dynamically significant factors is attempted. An analysis of this type has already been presented for the factors concerned in coronary flow (3). The present report will deal with the following subjects:

1. Improvement of the heart.
2. Spontaneous changes in coronary resistance.
3. Spontaneous changes in heart rate.
4. Adjustments of cardiac output.
5. Adjustments of peripheral systemic (and/or pulmonary) resistance.
6. Adjustments of heart rate.
7. Changes in pulmonary vascular resistance.

*Improvement of the Heart.* Improvement of the heart was seen frequently during the first 30 or 45 minutes after the preparation was established and sometimes later in the experiment. It was manifested, for instance, by a fall in the venous pressure on the right and/or left side of the heart at constant cardiac output and aortic pressure (figs. 6 and 11 of a previous report (3) and fig. 2 of a subsequent report (2)). There was, under these circumstances, a visible shrinkage in heart size. In other instances improvement was evident from an increase in cardiac output without a concomitant rise in left and/or right venous pressure. Where the improvement of the heart was seen just after the preparation was established, it probably represented recovery of a heart from the handling and manipulations necessary for setting up the experiment.

There is reason to believe that spontaneous increase in coronary flow, which occurs in some but not all cases within 30 to 45 minutes after setting up the

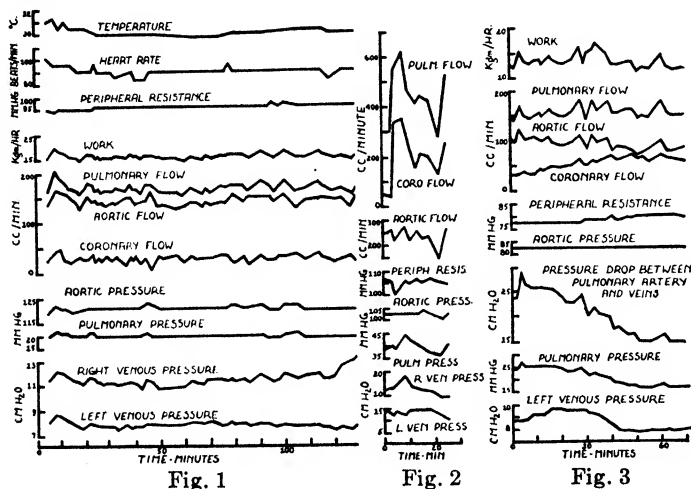


Fig. 1

Fig. 2

Fig. 3

Fig. 1. Chart showing pertinent data on a portion of a heart-lung experiment during which little variation occurred in the general level of the various variables measured. After 120 minutes progressive heart failure appeared, evidenced by a rise in right venous pressure without change in pulmonary flow or in the other variables. During the first portion of the chart a drop of  $1^{\circ}\text{C}$ . in blood temperature occurred, accompanied by a simultaneous drop in heart rate of about 10 beats/min. Except for right venous pressure after 120 minutes, the fluctuations in pulmonary flow are accompanied for the most part by like changes in the coronary and aortic flow and in the right and left venous pressures. *KgM.* is kilogram-meters in this and other figures. Discussed further in text of this and a subsequent report (2).

Fig. 2. Chart showing pertinent data on a portion of a heart-lung experiment prior to progressive heart failure to show lack of effect of large changes in cardiac output (pulmonary flow) on aortic pressure. Only in the first 8 minutes could this be attributed to adjustments of peripheral systemic resistance. After this the peripheral resistance was at a constant level. Despite the constancy of aortic pressure, coronary flow varied greatly and paralleled the changes in pulmonary flow. Similar changes, but of lesser degree were also induced, for the most part, upon the right venous and pulmonary arterial pressures. The left venous pressure like the aortic pressure was not affected by the changes in cardiac output. *Pulm.* is pulmonary, *Coro.* is coronary, *Periph.* is peripheral, *Press.* is pressure, *R.* and *L.* are right and left, *Ven.* is venous. Discussed further in text of this and a previous report (3).

Fig. 3. Chart showing pertinent data on a portion of a heart-lung preparation prior to progressive heart failure to illustrate the effects of spontaneous pulmonary and coronary dilatation. The latter led to the progressive rise in coronary flow at constant level of cardiac output (pulmonary flow), the aortic pressure remaining constant, later being prevented from falling by increasing the peripheral systemic resistance. The resulting redistribution of cardiac output between the aortic and coronary flows is apparent. Spontaneous pulmonary dilatation is evidenced by the drop in pulmonary arterial pressure and the decline in the pressure drop between the pulmonary artery and veins. The fluctuations in pulmonary flow are reflected, for the most part, in the pulmonary arterial pressure and in the pressure drop between the pulmonary artery and pulmonary veins, in the cardiac work and in the aortic and coronary flows. Discussed further in text of this and a previous report (3).

preparation (compare figs. 6 and 11 of a previous report (3)), may have been the main factor leading to improvement in cardiac condition. This presupposes

that in some of the preparations the coronary flow was at a critical level and the power of the heart limited by coronary flow. Evidence for this assumption is seen in some cases of delayed improvement following spontaneous increase in coronary flow.

*Spontaneous Changes in Coronary Flow.* The occurrence of spontaneous dilatation of the coronary bed has already been discussed in an earlier communication (3). Its presence was deduced from readings showing an increase in coronary flow with a concomitant decrease in aortic-vena cava flow and a tendency for a drop in aortic pressure. In this report we will deal with the effects of these changes upon the pressures and flows elsewhere. The most clear-cut information is obtainable from those heart-lung preparations in which cardiac output is kept constant, and we will confine our analyses to these. In order to maintain the aortic pressure constant, the artificial systemic resistance needed to be increased in most instances, since the reduction in coronary resistance tended to reduce the total systemic peripheral resistance. The adjustments, except in one case, were successful in maintaining aortic pressure. They led to a further decline in aortic flow and a further increase in coronary flow.

In a number of experiments in which coronary flow increased, aortic pressure did not fall even though the systemic peripheral resistance was not adjusted (cf. fig. 3, first period, and fig. 9 of a previous report (3)). The explanation for this probably lies in the fact that the calibre of the tubing constituting the artificial peripheral resistance is not only a function of the pressure exerted around it, but of that exerted within it. Recently it has been shown (5) that when the pressure inside a thin rubber tube is approximately equal to that outside it, small, almost immeasurable, changes in internal pressure will produce large changes in tube calibre. With a constant cardiac output, when coronary flow increases, aortic flow decreases. Associated with this decrease in aortic flow may be a small decrease in internal pressure in the tubing of the aortic-vena cava circuit. This may be too small to affect the aortic pressure noticeably but may be sufficient to alter considerably the calibre of the tubing in the artificial resistance, leading to an increase in the resistance offered by it. The pressure change in the air chamber surrounding the artificial resistance is too small to be measurable with a mercury manometer because the volume change produced is small. The increase in resistance so produced in the aortic-vena cava path may be sufficient, in some instances, to neutralize the decrease in coronary bed resistance so that *total* peripheral resistance, and aortic pressure, will not change appreciably.

Spontaneous coronary dilatation produced changes in pulmonary arterial and left and right venous pressures, which varied in different experiments. In all instances these effects were, however, only indirectly dependent on the coronary flow increase, being directly determined by the compensatory adjustment of the artificial systemic resistance, when this was necessary, or by the improvement in the state of the heart brought about by the improved blood supply, or, as will be discussed below, by simultaneous dilatation in the lung circuit.

The spontaneous change in coronary flow observed in these experiments could not be correlated with any change in arterial  $O_2$  content. Apparently, whatever humoral change in the blood occurred, causing dilatation, was independent of  $O_2$  content in the range found. Anoxemia of a degree sufficient to affect coronary flow was not encountered in these experiments.

*Spontaneous Changes in Heart Rate With and Without Temperature Alterations.* Some of the changes in heart rate encountered in these experiments depended on the blood temperature changes. In most experiments the temperature was constant within  $\frac{1}{2}^{\circ}C$ .; in one experiment it varied 2 or  $3^{\circ}C$ . The heart rate change with temperature averaged 10 beats/min. per degree (fig. 1). Aside from temperature variations, no great spontaneous variation in heart rate was found; rarely, the variations amounted to 10 beats per minute. No correlation could be established between these minor changes in heart rate and right venous pressure level, confirming the impression previously gained that changes in right auricular pressure are without effect on the rate of the pacemaker in the denervated preparation. By inference, therefore, the changes in heart rate reported in the intact animal must be reflex in origin (cf. 4).

*Effect of Adjustments of Cardiac Output.* The important influence cardiac output had on coronary flow has already been discussed in detail (3). Deliberate changes in cardiac output (pulmonary flow) were accompanied by a like change in one or both venous pressures (figs. 8 and 9 of a previous report (3) and fig. 1 of this report). In the heart-lung preparation the right venous pressure change was greater and more frequent than the left (cf. fig. 9 of a previous report (3)). The reason for this depends on the manner in which cardiac output was increased. In the heart-lung preparation this was done by increasing the total blood volume in the circuit. Just as further inflation of an already distended balloon would increase the pressure within by an amount depending upon the volume elasticity coefficient of the rubber, so would the increase in blood volume increase the pressure within the heart-lung circuit. The increase in pressure, assuming both ventricles to be able to cope adequately with the load, would not be the same in all parts of the circuit but would depend upon the elasticity characteristics of the various parts. In our artificial circuit the rubber tubing forming the systemic circuit apparently was more rigid than the vessels of the lung, for the right venous pressure rose more consistently and to a greater extent than left venous pressure. The fact that the pressure drop in the lung circuit (PP-LVP) did not increase in proportion to the increase of cardiac output, that is, that the resistance of the lung circuit decreased with increased cardiac output, is an anticipated consequence of the distention of these vessels by the addition of blood.

The aortic pressure likewise tended to rise when the cardiac output was increased (and fall when cardiac output fell (fig. 8 of a previous report (3))), but the artificial systemic resistance was often reduced to keep the aortic pressure constant (fig. 9 of a previous report (3)). In other instances where, without adjustment of the peripheral resistance, there was no noticeable rise in aortic pressure following increase in cardiac output (fig. 2 of this report and fig. 2 of a

subsequent report (3)) it was suggested (3) that in a distensible circuit the pressure tends to vary as some root (between the 1st and the 5th) of the cardiac output. Consequently, at high levels, aortic pressure would vary only slightly with large changes of cardiac output.

In the isolated heart preparation, the pulmonary as well as the systemic circuit consisted of rubber tubing. The mechanism for increasing cardiac output in this preparation consisted in increasing the left venous pressure. The aortic pressure was usually held constant despite the cardiac output increase by decreasing the systemic resistance. Consequently the pressure drop across the systemic resistance could vary not at all or in either direction. It was found, however, that the right venous pressure usually, but not always, rose with increasing cardiac output but to a lesser extent than did the left venous pressure. Furthermore, any tendency for pulmonary arterial pressure to rise in this preparation was neutralized by decreasing the pulmonary resistance.

Thus, it will be seen that while changes in cardiac output tend to produce like changes in the pressures of the preparations some of the latter changes are automatically minimized, and some are lessened by adjustment of the artificial peripheral resistance in the systemic circuit (and in the artificial pulmonary circuit in the isolated heart).

*The Effect of Changes in the Artificial Peripheral Resistances.* Changes in the artificial peripheral resistance of the systemic circuit were necessary to keep the total peripheral resistance constant and the aortic pressure level unaltered, when spontaneous coronary resistance changes occurred or when cardiac output had been changed deliberately. The effect of the procedure has been discussed earlier in this report and in a previous report (3). In several instances, the peripheral resistance of the systemic circuit (and of the pulmonary circuit) was deliberately changed in order to alter the work of the heart. The effect of changing systemic and pulmonary artificial resistances can best be analyzed when the cardiac output is kept unchanged.<sup>4</sup> The effect of changes in the systemic peripheral resistance on the partition of the cardiac output between the coronary and other beds has been dealt with at some length (3). It is important to re-emphasize that beyond a certain point, raising systemic peripheral resistance failed to raise aortic pressure, although it continued to divert more of the cardiac output into the coronary circuit. As we have shown (3) pressure in the vessels, the coronaries in the case at hand, varies as some root (between the first and the fifth) of the volume flow. Obviously at high flow levels, the increment in pressure is extremely small for a given increase in flow. It was found that in such cases change in the pulmonary arterial and venous pressures was usually minimal or absent (figs. 7 and 10 of a previous report (3)). When the aortic pressure was raised, the rise was usually transmitted backward and produced a like change in

<sup>4</sup> Here we are not discussing immediate transitory changes in the pulmonary flow measurement due to redistribution of blood between the parts of the circuit which occur when the systemic peripheral resistance is adjusted, and which disappear as soon as the redistribution is completed. This immediate effect was a decrease in the pulmonary flow measurement when the systemic peripheral resistance was manually increased (fig. 2 of a subsequent report (2)), and the reverse when this resistance was decreased.

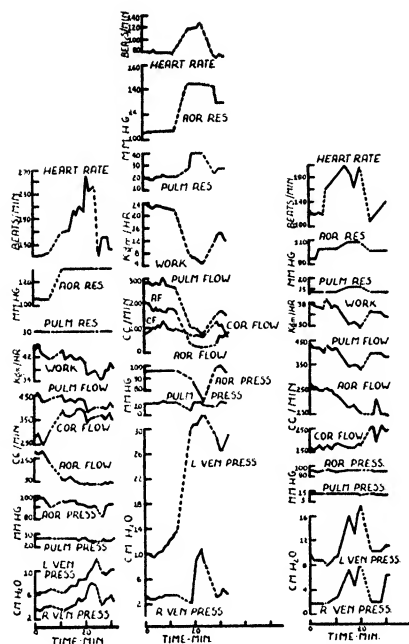


Fig. 4

Fig. 5

Fig. 6

Fig. 4. Chart showing pertinent data on a portion of an isolated heart experiment in which heart rate was artificially varied with a Lewis interruptor. Progressive heart failure is manifest toward the end by the higher level in right and left venous pressure when the heart rate is again slowed compared to the first period of slow heart action, although the pulmonary flow is at about the same level in both periods. During the period of rapid heart rate, cardiac output fell despite constant level of the reservoir from which the left heart filled. The decline in aortic pressure was minimized by increasing the aortic peripheral resistance. This latter resulted in a sharp increase in coronary flow by diversion despite the fall in aortic pressure, and the aortic flow declined. The rise in venous pressures at the same time that cardiac output fell results from the sharp reduction in the period of filling of the ventricles as the heart is accelerated, and should be distinguished from the lack of fall in venous pressure later when the heart slows again which is due to progressive heart failure. The dotted lines in this and later figures are used to represent periods when no readings were taken. In this and subsequent figures, *Aor.* is aortic, *Res.* is resistance, *Pulm.* is pulmonary, *Press.* is pressure, *L.* and *R.* are left and right, *Ven.* is venous, *Cor.* is coronary. Discussed further in text of this and a previous (3) and subsequent report (2).

Fig. 5. Chart showing pertinent data on a portion of an isolated heart experiment in which the heart rate was artificially varied with a Lewis interruptor. Progressive heart failure is present as evidenced by comparing the two periods of slower heart rate which reveal a reduction in cardiac output (pulmonary flow) in the later period, although the level of the reservoir from which the left heart filled remained constant, and a marked rise in left and a slighter rise in right venous pressure. During the period of temporary induced cardiac acceleration, pulmonary flow fell sharply despite the constant pressure level of the inflow reservoir, at the same time the venous pressures rose sharply. These changes, indicative of the reduced time available for filling as the heart is accelerated, are to be distinguished from those of progressive heart failure discussed above. Pulmonary arterial pressure was maintained constant by increasing the pulmonary peripheral resistance. A similar and more marked increase in aortic peripheral resistance failed to prevent a drop in aortic pressure. In this experiment, unlike the last (fig. 4), the drop in cardiac output

left venous pressure and in pulmonary arterial and right venous pressure (fig. 9 of a previous report (3)), although the changes in the latter were of much smaller degree and were often neutralized or reversed by other simultaneous changes (cf. fig. 9 of a previous report (3)). The extent of elevation of left venous pressure and pulmonary arterial pressure caused by elevated aortic pressure appeared to depend on the power of the left heart and the extent of elevation of right venous pressure by elevated pulmonary arterial pressure appeared to depend upon the power of the right heart. Occasionally, increasing the systemic peripheral resistance led to a sustained decrease in cardiac output in the heart-lung although the blood volume had not been altered; apparently the heart was in these instances unable to raise its energy expenditure to overcome the increased resistance load.

In the heart-lung preparation, especially when the aortic pressure was not affected, increasing the systemic peripheral resistance often caused a drop in the pressures beyond the artificial resistance, i.e., right venous pressure and usually pulmonary arterial pressure (fig. 9 of a previous report (3) and fig. 2 of a subsequent report (2)). Thus, a rise in peripheral resistance operated to raise the pressures upstream and lower those downstream much as a dam would. In distant parts of the closed heart-lung circuit these two oppositely directed effects tend to neutralize each other. Sometimes the backward directed effect dominates, at other times the forward directed effect dominates.

When the pulmonary resistance of the isolated heart was altered deliberately it caused a similar change in pulmonary arterial pressure (fig. 11 of a previous report (3) and fig. 5 of this report), whereas, as we have seen, due to the existence of the alternative coronary pathway with its variable resistance, a change in aortic pressure is not always produced by changes in systemic resistance. A similar change in right venous pressure also occurred, the extent depending apparently on the power of the right heart. Moreover, changes in pulmonary resistance in the isolated heart usually caused similar changes in the aortic pressure apparently secondary to the rise in right venous pressure.

*Adjustments in Heart Rate.* The effect of heart rate changes on coronary

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dominated over the increase in aortic peripheral resistance, with the result that coronary flow fell slightly, but not to the extent of the aortic flow or the cardiac output. *CF* is coronary flow, *AF* is aortic flow. Discussed further in text of this and a previous (3) and subsequent report (2).

FIG. 6. Chart showing pertinent data on a portion of an isolated heart experiment in which the heart rate was artificially varied with a Lewis interruptor, prior to heart failure. In this experiment spontaneous coronary dilatation accounts for the greater coronary flow after the period of cardiac acceleration compared to that before this period. In this experiment, like the last two, cardiac output (pulmonary flow) is an inverse function of the heart rate even though the level of the filling reservoir is unchanged. Again these changes in cardiac output are due to alterations in the time for ventricular filling, and this accounts for the changes in the venous pressures. The coronary flow is little affected by the cardiac acceleration in that there is no apparent deviation of the rising flow curve dependent on the spontaneous coronary dilatation. Apparently the opposite influences of increase in aortic peripheral resistance and decreased cardiac output neutralize each other. Discussed further in text of this and a previous report (3).

flow has already been discussed (3) and it was shown that the alterations in coronary flow were variable depending secondarily upon the changes in cardiac output and peripheral resistance adjustment following the heart rate alteration. The change in aortic pressure was also shown to be the resultant of these two factors. Examination of figures 4, 5 and 6 will show that, except as noted below, the changes in pulmonary arterial, left and right venous pressures are also readily attributable to the adjustments in cardiac output and peripheral resistances following upon the heart rate change, just as was the case when cardiac output and peripheral resistance were changed without change in heart rate.

It therefore is not necessary to dwell on the secondary change in these variables but to consider what effect heart rate had on cardiac output. In the isolated heart experiments in which these studies were made, no attempt was made to keep cardiac output constant. Whenever heart rate sped up, cardiac output fell, apparently because the time for filling was so encroached upon that the resulting stroke output declined more than the heart rate increased (figs. 4, 5 and 6). The decreased cardiac output tended to cause aortic and pulmonary arterial pressure to fall and left and right venous pressure to rise, since the blood not pumped by the ventricles tended to accumulate on the venous side (fig. 5). Often aortic and pulmonary arterial pressure were restored to normal by increasing the artificial peripheral resistances so that only the left and right venous pressure rise remained (figs. 4 and 6). It will be seen that a decrease in cardiac output without a decrease in circulating blood volume leads to like changes in arterial and an opposite effect on venous pressure, whereas changes in cardiac output due to changes in blood volume led to like changes in the venous and arterial pressures. A decrease in cardiac output resulted not only when the heart rate was increased beyond the optimum, but too great a slowing of the heart rate also caused a fall in cardiac output and resultant changes in pressures because of the decreased rate of filling in the later part of diastole.

In evaluating the significance of venous and arterial pressures with marked changes in heart rate in the intact animal, this purely mechanical effect of lack of filling time as the heart accelerates or lack of sufficient increase in filling per beat as the heart rate becomes very slow must be recognized and distinguished from the effects of heart failure.

*Changes in Pulmonary Resistance.* Pulmonary resistance in the isolated heart preparation was artificial and therefore subject to deliberate changes only. In the heart-lung preparation, three sets of circumstances were apt to change the resistance of the pulmonary circulation: *a*, spontaneous vasodilatation similar to that observed and described for the coronary vessels; *b*, resistance changes caused by changes in flow and pressures elsewhere in the circuit, and *c*, changes caused by pulmonary edema or engorgement.

Spontaneous decreases in pulmonary vascular tone often appeared, evidenced by a drop in pulmonary arterial pressure with a simultaneous decrease in the drop from pulmonary arterial to left venous pressure (*PP-LVP*, fig. 3), and usually reflected in a fall in right venous pressure. This was commonly asso-



ciated with a simultaneous decrease in coronary circuit resistance (fig. 3) and is ascribed to the same hypothetical cause, the accumulation of vasodilator substances in the circulating blood.

Changes in pulmonary resistance secondary to pulmonary flow have already been referred to in the course of the previous discussion and will be briefly recapitulated here. Thus, calculation of the change in pressure drop and comparison with change in pulmonary flow shows, as expected, that when pulmonary flow increases the pressure drop does not tend to increase proportionally. It must therefore be concluded that the decreased resistance is brought about by passive dilatation of the pulmonary vessels when pulmonary flow increases.

Changes in pulmonary resistance, like changes in systemic resistance, have the effect of causing the pressures upstream to change in the same direction while the changes in pressure downstream go in the opposite direction. The distance over which these two opposite effects are transmitted is variable, and so the effect on the pressure at any distant point is variable. In the isolated heart only upstream pressure changes occur when the artificial pulmonary resistance is altered since the circuit between the pulmonary artery and pulmonary veins is open. In the intact circulation, the large capacity of the systemic circuit will tend to prevent these pressure effects from being transmitted beyond the peripheral systemic vessels.

Pulmonary edema preceding heart failure was only rarely observed in the heart-lung preparation after an initial period of trial and error during which careful methods of preparing the blood were worked out. In the series of experiments analyzed in this study, there was only one case of frank pulmonary edema preceding other symptoms of heart failure. Milder degrees of edema were occasionally encountered. It was our experience that it is more apt to occur with blood which shows a tendency to foam.

When great care in the preparation of the blood was taken, the formation of emboli or thrombi was very rare. The occasional plugging up of minor pulmonary vessels was not followed by pulmonary edema. It was found that such plugging had no effect on the flows and pressures. This is to be expected in view of the numerous parallel "pathways" through the lungs. When one is occluded, its blood is diverted to the others. Even plugging larger pathways need not lead to an increase in pulmonary resistance. In an earlier report (3) it was shown that increases in extracoronary resistance tend to decrease, by passive dilatation, the resistance in the coronary path, often leaving the total resistance unchanged. Similarly, total pulmonary resistance may remain unaltered even if some of the pathways are no longer open.

In the few cases in which pulmonary edema was observed, it was found that in order to maintain the cardiac output and aortic pressure constant, blood had to be added and the artificial systemic peripheral resistance had to be increased. Eventually these measures failed to maintain the cardiac output and the aortic pressure. During the period when cardiac output and aortic pressure could be maintained constant, the most striking consequence of pulmonary edema was a rise in the pulmonary arterial pressure. The pressure drop between pulmonary

arterial and venous pressures increased as would be expected when the resistance between these two points increases. On the other hand the rise in pulmonary arterial pressure led, after a lag, to a rise in right venous pressure, because the increased pulmonary resistance imposed an increased load on the right ventricle. When cardiac output and aortic pressure could no longer be maintained the pressures and flows elsewhere were influenced by the latter changes.

#### SUMMARY

In order to determine how many of the changes occurring in the course of heart failure are attributable directly to the loss of power of the heart, and how many represent spontaneous change or those induced by manipulation, the constancy and interrelation of the various pressures and flows in the period of little or no failure in 13 isolated hearts and 25 heart-lung preparations are considered.

Improvement of the heart was frequently seen and manifested by a fall in venous pressure with constant cardiac output and work, or by an increase in cardiac output without rise in venous pressure. Improvement of the heart occurred early and apparently represented recovery from the initial manipulations in setting up the preparation; it also occurred later and in these cases, as in others, a common factor apparently was improvement in coronary flow.

In the course of a number of experiments, a spontaneous decrease in coronary resistance led to increase in coronary flow, and since the total peripheral resistance tended to decline, aortic pressure tended to fall. Usually peripheral resistance was adjusted to keep up aortic pressure. In a number of experiments the aortic pressure did not fall even though the systemic peripheral resistance was not adjusted. The probable explanation for this is discussed. The effects of spontaneous coronary dilatation upon the pulmonary arterial and the venous pressures varied and were actually determined by coincidental adjustments or changes. The spontaneous changes in coronary flow could not be correlated with variations in arterial oxygen content; apparently anoxemia of a degree sufficient to affect coronary flow was not encountered in these experiments.

Almost all the spontaneous changes in heart rate were related to temperature change and averaged about 10 beats per minute per degree centigrade. No correlation between heart rate and right venous pressure levels could be established.

Deliberate changes in cardiac output were accompanied by a like change in one or both venous pressures. In the heart-lung preparation the right venous pressure change was greater and more frequent than left. In the isolated heart preparation the left venous pressure rise was more frequent and greater than the right. Aortic pressure tended to rise but not in all cases. The cause for these changes is discussed.

When changes in the artificial systemic resistance did not change the aortic pressure, change in the other pressures was usually minimal or absent. When the aortic pressure was elevated by this procedure left venous pressure usually rose. A rise was seen less frequently and to a lesser extent in the pulmonary arterial and right venous pressures. Contrary effects of the increase in peripheral

resistance operating downstream sometimes neutralized or reversed the pressure changes on the right side of the heart. Increase in artificial aortic resistance operated to raise the pressures upstream and lower them downstream.

Alteration in the pulmonary resistance in the isolated heart preparation caused changes in the same direction in pulmonary arterial pressure, right venous pressure and usually aortic pressure.

Apart from the effect of secondary adjustments when the heart rate was changed, increase or decrease in heart rate beyond the optimum for adequate filling decreased the cardiac output. Arterial pressures tended to fall and venous pressure to rise and the rise in venous pressures was accentuated by adjustments in peripheral resistances to maintain the arterial pressures.

The development of pulmonary edema, but not the plugging up of minor vessels by thrombi (both rare phenomena), increased the pulmonary resistance, increased the pulmonary arterial to venous pressure drop, and led to an increase in right venous pressure.

Spontaneous decreases in pulmonary resistance in the heart-lung, usually associated with simultaneous decrease in coronary resistance, decreased the pressure drop from pulmonary artery to vein and were usually reflected also in a fall in right venous pressure. Increase in the pulmonary flow by distending the lung blood vessels in the heart-lung tended to lessen the expected increase in the pressure drop between the pulmonary artery and veins.

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# THE DYNAMIC ALTERATIONS IN HEART FAILURE IN THE ISOLATED HEART AND HEART-LUNG PREPARATION<sup>1</sup>

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Spontaneous heart failure has been studied by several investigators (1-9) and in this laboratory (10, 11, 12) using the isolated heart and heart-lung preparations in which failure develops at varying periods following removal of the heart from the body. Barring the occurrence of accidents or ventricular fibrillation, the development of progressive heart failure terminates such experiments. The time of its occurrence and the rate of its development in the isolated heart and heart-lung preparations which we have employed have been discussed and compared in the two preparations (13). As was stated, the causes for the development of failure are a matter of speculation. It was found (13) that in the heart-lung preparation failure develops later than in the isolated heart preparation, and that in preparations with heparinized blood failure develops later than in preparations with defibrinated blood. Some alteration in the circulating blood, prevented or cancelled by other body organs in the intact animals, is held responsible for the development of failure. As evidence for this, it is worthy of note that in some instances we have found hearts in severe failure to show temporary improvement when the blood was drained from the system and replaced by unused blood. The suggestion has been made that in some cases coronary insufficiency due to small coronary vessel thrombi is responsible (14) but this was not apparent in any of our preparations. Coronary flow was found to remain constant or to increase during heart failure; when a decrease was observed it could be accounted for on the basis of factors which have been demonstrated to cause coronary flow to decrease in the *non-failing* heart.

Spontaneous heart failure in the isolated heart or heart-lung preparation need not be related to heart failure occurring in the intact animal or man. Nevertheless, certain information can be obtained from these preparations which helps to clarify some of the changes occurring in clinical heart failure. More important, it is necessary to evaluate the dynamic changes which occur in spontaneous heart failure in these preparations in order to separate those changes which can be ascribed *directly* to heart failure from those which are *independent* or *secondary* and not peculiar to heart failure, that is, from changes observed following spontaneous or induced alteration of factors controlling the cardiodynamics in the non-failing heart. The latter have been discussed in previous communications (15, 16).

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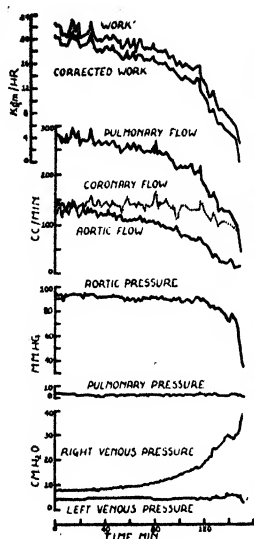


Fig. 1

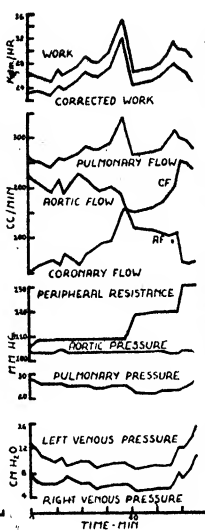


Fig. 2

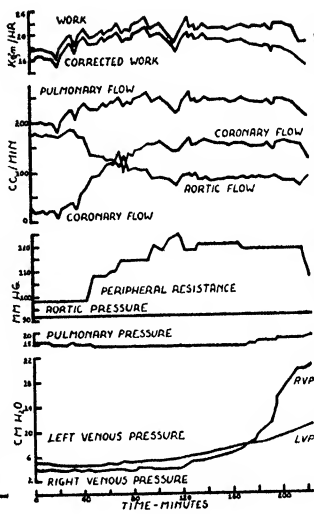


Fig. 4

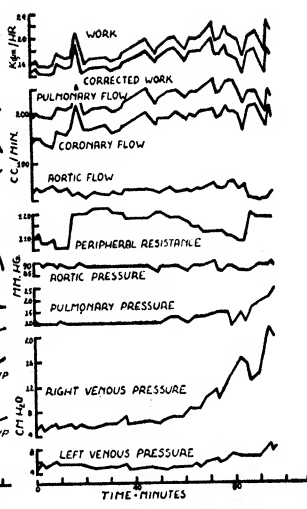


Fig. 5

Fig. 1. Chart showing pertinent data on an isolated heart preparation in which slight left and severe right "congestive" failure and severe "forward" failure developed and eventually terminated the experiment. No attempt was made to keep work constant in this experiment. The blood reservoir pressure level and the arterial artificial resistances (not shown) were constant, not being adjusted during the entire experiment. At 20 minutes "forward" failure began, evidenced by decreasing pulmonary flow which continued to fall at an accelerating rate. Except for minor changes, aortic pressure did not begin to decrease until 87 minutes, but then fell at an increasing rate, eventually precipitously. Pulmonary arterial pressure, already low, fell only terminally. Right "congestive" failure began at 24 minutes, evidenced by a rising right venous pressure; the rise progressively increased in rate and was eventually very rapid. Left venous pressure was surprisingly constant. Aortic flow fell with pulmonary flow. Coronary flow rose early due to a spontaneous coronary dilatation but levelled off and eventually decreased as pulmonary flow decreased. Work paralleled pulmonary flow until the decrease in aortic pressure became significant. "Corrected work" was less than work by a percentage difference which increased as the venous pressures rose with "congestive" failure. However, the decrease in pulmonary flow, with "forward" failure, reduced the absolute difference between work and "corrected work." In this and subsequent figures KgM. is kilogram-meters. Discussed further in text of this and previous reports (15 and 16).

Fig. 2. Chart showing pertinent data on a heart-lung preparation which developed severe left and right "congestive" failure. Work was kept as constant as possible by maintaining pulmonary flow and aortic pressure level by adjustment of blood volume and peripheral resistance where necessary; control of pulmonary flow, however, was rough. During the early portion of the experiment both venous pressures declined with improvement of the heart; at 42 minutes they began to rise, with the onset of left and right "congestive" failure, and the rise rapidly became steep. Marked spontaneous coronary dilatation occurred throughout the experiment, and at two points peripheral resistance was increased to prevent aortic pressure from falling; each increase further increased the coronary flow and decreased the aortic flow; the first increase also caused right venous pressure, pulmonary arterial pressure and left venous pressure to fall. Pulmonary pressure eventually rose, secondary to the rise in left venous pressure. Work paralleled pulmonary flow but as the venous pressures rose the difference between "corrected work" and work as ordinarily calculated increased.

The methods of establishing the preparations, the methods of making the measurements and the adjustments employed have been described (13).

*Criteria of progressive heart failure.* In reviewing the instances of progressive heart failure in the isolated heart and heart-lung preparations, we have come to the following conclusions concerning the criteria of progressive heart failure:

a. In those experiments in which the calculated work of the heart was maintained constant by maintaining the cardiac output and the arterial pressures constant, progressive failure was manifested by an accelerated rise in left or right venous pressure (figs. 2, 3 and 4 of this report and fig. 3 of a previous report (12)); toward the end, in addition, a drop in cardiac output and/or aortic pressure could not be prevented in some.

b. In those experiments in which the work was *not* kept constant, progressive failure was often manifested by an accelerating decline in cardiac output and/or aortic and pulmonary arterial pressures (fig. 1), and the decline of the latter

This introduces an element of "forward" failure, obscured by the irregularity of pulmonary flow. The fluctuations in pulmonary flow are reflected for the most part by like changes in aortic and coronary flow, and in pulmonary arterial pressure and venous pressures. *AF* is aortic flow; *CF*, coronary flow. Discussed further in text of this and previous reports (15 and 16).

Fig. 3. Chart showing pertinent data on a heart-lung preparation which developed severe left and right "congestive" failure and eventually "forward" failure. Work was controlled in the same manner as in the experiment illustrated in figure 2. Terminally, in spite of increase in blood volume, it was impossible to maintain pulmonary flow, which began to decline evidencing "forward" failure. Left "congestive" failure, with left venous pressure rising at an accelerating rate, began at 54 minutes; right "congestive" failure began at about 116 minutes; the right venous pressure curve was eventually flattened out by "forward" failure. Changes in pulmonary flow were mirrored by similar changes in coronary flow; in addition, spontaneous coronary dilatation is also evident in the rising slope of coronary flow in the first half of the experiment; this rise was accentuated by increases in peripheral resistance necessary to keep aortic pressure from falling as coronary resistance decreased. Aortic pressure was kept level; pulmonary arterial pressure eventually increased as left venous pressure rose. Work paralleled pulmonary flow, but "corrected work" distinctly decreased as the venous pressures rose. The fluctuations in pulmonary flow are reflected for the most part by like changes in aortic and coronary flow but the venous pressures show similar fluctuations only infrequently. Discussed further in text of this and a previous report (16).

Fig. 4. Chart showing pertinent data on a heart-lung preparation which developed moderately severe left and severe right "congestive" failure. Work was controlled as in the other heart-lung experiments illustrated, but control of pulmonary flow was imperfect, with an increasing trend during most of the experiment. The initial rising trend in venous pressures was due to a pulmonary flow rise; rise in left venous pressure due to "congestive" failure began at 53 minutes and that in right venous pressure at about 23 minutes. Aortic pressure was fluctuant but level. Pulmonary arterial pressure rose with rise of left venous pressure, but the rise was so marked as to indicate increasing pulmonary resistance due to pulmonary congestion and/or edema. Coronary flow paralleled pulmonary flow; the changes in peripheral resistance were not marked enough to discernibly affect coronary flow. Work paralleled pulmonary flow, but when the venous pressure rose "corrected work" diverged from work as ordinarily calculated. Most of the fluctuations of pulmonary flow are reflected by similar changes in aortic and coronary flow, and in arterial and venous pressure. Discussed further in text of this and previous reports (15 and 16).

could not even be prevented by increasing the artificial peripheral resistance in the systemic (and pulmonary) circuit (fig. 2 of a previous report (10)).

Since venous pressure remained constant when the heart was in a steady state as long as the arterial pressures, peripheral resistances and cardiac output were kept constant (fig. 1 of a previous report (16)), a significant rise of venous pressure under such conditions was interpreted as indicating a loss of power of the heart, that is, as indicating that the heart had to be distended more to maintain the same output and work level. The cardiac output was maintained in the isolated heart by raising the pressure level of the reservoir feeding the left auricle, and in the heart-lung by increasing the circulating blood volume. Both procedures ensured higher venous pressure levels.

In our studies, determination of cardiac output (and incidentally of cardiac work) is based on measurements of pulmonary flow. It must be emphasized again that the pulmonary flow is a reliable measurement of cardiac output while the aortic flow below the mouths of the coronary vessels is not. The latter is influenced by changes in coronary flow, and in these experiments, spontaneous coronary dilatation of a degree sufficient to increase coronary flow and to decrease aortic flow was frequently observed (15). The employment of aortic flow as an index of cardiac output is, therefore, not justified in such preparations, and its use by some for this purpose would require reexamination of results so derived.

In the heart-lung preparation the attempt was always made to keep the cardiac output constant by the addition of blood to the system whenever cardiac output declined. This was done because in the closed-circuit preparation employed one cannot be sure of a constant rate of return to the heart even when the circulating blood volume is left unchanged. Moisture may be lost in the lungs and minute hemorrhages may occur; the dilatation of the heart in failure may increase the capacity of the circuit, as would spontaneous dilatation of the coronary and pulmonary vascular beds occurring at this time. Thus, the assumption that any decrease in cardiac output indicates failing power of the heart is unjustified. Only the terminal stages of failure in the heart-lung when it was no longer possible to maintain cardiac output by the addition of blood can be used to study the events following decline of cardiac output due to heart failure. With a constant pressure reservoir, as used in the isolated heart, these errors are absent; a decrease in cardiac output in this preparation may be taken to be a sign of loss of power of the heart.

The venous pressures as measured are an indication of the respective auricular pressures, which in turn are an index of the initial pressures in the respective ventricles just before the beginning of systole. However, in the failing heart the right and left venous pressures are not entirely satisfactory indices of the degree of distention of the ventricles because as failure develops the tonus of the ventricles declines (18, 19). The degree of dilatation thus may be higher than is indicated by the venous pressures, and it is possible that in some cases the venous pressure rise may be minimal for a time because of the tonus change. As a ventricle distends, its output should increase in accordance with Starling's law,

hence failure of this to occur suggests the development of loss of ventricular power. The corollary also follows that when the output of the heart is maintained constant and the venous pressure rises, this too is an indication of loss of power of the heart, since to maintain the output at its set level the heart has to be distended.

When venous pressures rise, we are dealing with "congestive" heart failure (figs. 2, 3, 4 of this report, fig. 3 of a previous report (12), figs. 1 and 4 of another (16) and figs. 7 and 11 of a third (15)); when the cardiac output declines and the arterial pressures fall we are dealing with "forward" failure, and when both occur we are dealing with "mixed" failure (fig. 1 of this report, fig. 2 of a previous report (10), fig. 11 of another report (15) and fig. 5 of a third (16)). Heart failure, therefore, may be "congestive," "forward" or "mixed," and it may be mainly right (figs. 3 and 4) or mainly left (fig. 2 of a previous report (10)). As it progresses heart failure usually ends as a "mixed" form whether it begins as "congestive" or "forward" failure. In addition, the heart failure may shift from a dominant right to a dominant left or vice versa. Criteria to judge the degree of congestive failure developed are rough, permitting classification of the failure as slight, moderate or severe, as judged by the slope of the venous pressure curve at the time when the greatest severity of failure was present.

In applying these definitions to our preparations, it must be remembered that heart failure was not usually allowed to take a spontaneous course. Whenever the attempt was made to keep the work level constant, as in all the heart-lung and in the later isolated heart experiments, incipient or actual "forward" failure was converted to "congestive" failure. This was done by compensatory adjustments of peripheral resistance and of blood volume or pressure level just beyond the reservoir, such as to keep arterial pressures and cardiac output as constant as possible. Only when these adjustments failed, in the terminal phase of such experiments, was forward failure manifested.

On the other hand, in the early isolated heart experiments adjustments to keep work constant were not made. In these experiments, with the pressure level from the reservoir kept constant, "forward" failure could readily occur; actually, as will be discussed later, all such experiments showed "forward" failure.

*Failure in isolated heart preparation.* Progressive heart failure was observed in 22 preparations. In 13 early experiments, comprising group I, the pressure level from the reservoir was kept constant and the work of the heart allowed to change spontaneously; in 8 of these the peripheral resistances were adjusted to keep the arterial pressures as constant as possible. In a second group, group II, comprising 9 later experiments, work was kept as constant as possible. Two additional facts concerning these experiments must be appreciated: 1, in 13 preparations, heart failure was not permitted to continue uninterrupted; interruption occurred by accident or by the introduction of a drug; 2, in 7 experiments failure of a combined type was present at the time readings were begun so that it was not possible to determine which form was initially present. The



following is a breakdown of the forms of heart failure seen:

	Total	Group I	Group II
a. Right "congestive" failure.....	1	0	1
b. Left "congestive" failure.....	1	0	1
c. Combined left and right "congestive" failure.....	6	0	6
1. of which the congestion was dominant in the right.....	1	0	1
2. of equal degree in the two sides.....	5	0	5
d. "Mixed" form of heart failure.....	14	13	1
1. consisting of left "congestive" and "forward" failure.....	4	4	0
2. consisting of combined left and right "congestive" and "forward" failure.....	10	9	1
I. of which the congestion was dominant in the left.....	7	6	1
II. of equal degree in the 2 sides.....	3	3	0

In these experiments, left "congestive" failure occurred in all but one instance, right "congestive" failure was absent in 5 cases, and "forward" failure was absent in 8 cases; the absence of "forward" failure was noted only in group II. In 7 preparations progressive failure was present from the onset, in the other 15 it developed in the course of the experiment.

The onset of failure began as:

	Total	Group I	Group II
Left "congestive" failure in.....	4	1	3
Right "congestive" failure in.....	2	0	2
"Forward" failure in.....	1	0	1
Left and right "congestive" failure in.....	8 (4*)	4 (2*)	4 (2*)
Left "congestive" and "forward" failure in.....	4 (2*)	4 (2*)	0
Combination of all three forms in.....	3 (1*)	3 (1*)	0

\* Figures in ( ) refer to number of experiments in which failure was present when readings were begun.

It is interesting to note that the experiments showing "forward" failure in combination with "congestive" failure were shorter in duration than those showing "congestive" failure alone. The development of "forward" failure seems to set up a vicious mechanism, presumably through its effects on coronary flow, which hastens termination of the experiment.

*Variations of the individual pressures and flows during progression of heart failure in the isolated heart. a. Arterial pressures.* Aortic pressure fell in half the cases with "forward" failure (fig. 1). Of the 7 cases in which aortic pressure did not fall with "forward" failure (fig. 5 of a previous report (16)), 6 were not permitted to continue until failure had fully developed. In the 7 cases in which aortic pressure fell, it tended to remain constant for a time after cardiac output had begun to decline (fig. 1 of this report and fig. 2 of a previous report (10)). Pulmonary flow fell without an aortic pressure drop, or before it dropped in 11 out of 14 cases. This occurred (fig. 1) even when the systemic peripheral resistance was not increased or not increased until later.

The reason for the constancy or delayed fall of aortic pressure is probably, as pointed out in a previous report (15), that there exists a critical level of cardiac

output and arterial pressure, at which changes in the cardiac output cause no measurable alteration in aortic pressure (and pulmonary arterial pressure). In the early stages of "forward" failure, therefore, declines in cardiac output may be unaccompanied by measurable alterations in aortic (and pulmonary arterial) pressure until the output drops below this critical level. This mechanism for maintaining aortic pressure may apply to the coronary flow as well as to the aortic-vena cava flow. Reduction of flow may bring about a reduction in diameter and consequently a considerable increase in resistance.

Increase of artificial resistance is effective in maintaining aortic pressure when it begins to fall, but as "forward" failure becomes more severe, it will fall at an accelerated rate in spite of all attempts to maintain it, since by this time a point in the cardiac output/arterial pressure curve is reached at which small changes in cardiac output produce large changes in arterial pressure.

These facts are of considerable implication for the intact circulation in that they point to the observation that a drop in arterial pressure is a sign of advanced rather than early "forward" failure. In short, "forward" failure can occur without a drop in arterial pressure not only because of neurogenic and other homeostatic mechanisms but probably also because of 1, a purely mechanical set of readjustments accompanying a decrease in cardiac output, and 2, the peculiar relationship that cardiac output has to aortic pressure such that above a critical level alterations in cardiac output cause no measurable variations in aortic pressure. Reliance on arterial pressure alone to judge "forward" failure is thus not as valuable as reliance on cardiac output measurements. The clinical implications are evident.

In "forward" failure, pulmonary arterial pressure behaved very much like aortic pressure (fig. 2 of another report (10)) presumably for the same reason (i.e., because of the cardiac output/arterial pressure relationship). The declines of aortic and pulmonary arterial pressure were often not simultaneous or parallel in degree. In "congestive" failure without "forward" failure pulmonary arterial pressure remained constant (fig. 11 of a previous report (15) and fig. 4 of another report (16)). Left "congestive" failure did not lead to an elevation in pulmonary arterial pressure since the pulmonary arteries were separated from the left heart by the inflow reservoir. The changes in pulmonary arterial and aortic pressure, therefore, are not peculiar to heart failure since they are seen also in decreases in cardiac output in non-failing hearts.

*b. Venous pressures.* In the typical case of right "congestive" failure, right venous pressure rose at an accelerating rate (fig. 1). However, when "forward" failure also developed, the latter prevented the acceleration of the rise of right venous pressure. In a few cases with "forward" failure, right venous pressure remained constant or actually fell when pulmonary arterial pressure fell markedly.

These changes in right venous pressure are not difficult to explain. Right venous pressure in the non-failing heart is a function of cardiac output and pulmonary arterial pressure, the two factors determining the work load of the right heart. Right venous pressure tends to fall when cardiac output declines and also when pulmonary arterial pressure falls. The effect of cardiac output

on right venous pressure is the more striking and constant of the two. In fact, when cardiac output is plotted against right venous pressure during the course of an experiment, reasonably good correlation is shown. If this curve is constructed for the case where the right heart is failing it will differ from the curve of the non-failing heart, in that at a given cardiac output the right venous pressure will tend to be higher, and at a given right venous pressure the cardiac output will tend to be lower. The change of right venous pressure when heart failure occurs will therefore depend upon the degree of loss of cardiac power as well as upon the presence and degree of any change in cardiac output.

The linear relationship between cardiac output and right venous pressure in the non-failing heart is due to the fact that, under constant conditions especially of heart rate, right venous pressure determines cardiac diastolic size and therefore cardiac output. When the heart fails the cardiac tonus may decline (18, 19) so that for a given cardiac output the right venous pressure would be lower. The more distensible heart which the loss of tonus produces can accommodate the blood not expelled when its output declines (because of loss of power) with a smaller rise of right venous pressure than the heart with normal tonus.

Right venous pressure is a simultaneous index of the load of the heart and of its power. A decline in power and an increase in load both cause it to rise, and the reverse will cause it to fall. Hence in judging whether a right venous pressure rise is an indication of right heart failure, the effect of any change in load as judged by cardiac output and pulmonary arterial pressure must first be discounted. The influence of loss of tonus of the ventricle as it fails also must be taken into account, but is probably not a major factor.

In the typical case of left "congestive" failure, left venous pressure rose at an accelerating rate. Stabilization of the venous pressure upon the onset of "forward" failure occurs for left venous pressure (fig. 1) as it does for right, and for the same reasons.

One further factor complicates the left venous pressure changes in our isolated heart preparation, and that is the presence of the reservoir from which the filling of the left heart may be controlled. In those experiments in which the pressure level just beyond the reservoir was not altered, and in which this pressure level was close to the head of pressure in the reservoir, any tendency for left venous pressure to rise as heart failure developed would be prevented. The pressures in the veins obviously could approach but not exceed that within the reservoir. The presence of the fixed reservoir in the experiments in which no adjustments were made thus acted to buffer the left venous pressure changes.

This limiting of left venous pressure elevation obviously is the reason for the development of "forward" failure in the early isolated heart preparation in which no adjustments of the pressure level from the reservoir were made.

Another factor which may contribute to the rise in venous pressures in the terminal stages of heart failure is the development of a relative mitral and/or tricuspid regurgitation when the heart is markedly distended, permitting backward transmission of the higher ventricular pressure.

*c. Pulmonary, aortic and coronary flows.* When pulmonary flow was arti-

ficially maintained constant during the course of heart failure the distribution of cardiac output between coronary flow and aortic flow was not altered in any way peculiar to heart failure. Thus, spontaneous coronary dilatation occurred during the development of heart failure as well as in its absence. In only one case was there a "spontaneous" decrease in coronary flow, possibly due to coronary thrombosis; the decrease began before failure developed and continued into the failure period. Otherwise, no change in coronary or aortic flow occurred in the absence of "forward" failure except when the systemic peripheral resistance was readjusted and here the changes were exactly like those in the non-failing heart.

In the presence of "forward" failure, coronary flow decreased as cardiac output fell (fig. 1), as might have been expected from the relationship shown to exist between cardiac output and coronary flow in the non-failing heart. In some instances of early or slight "forward" failure, coronary flow decreases were small or absent (fig. 1), in a few coronary flow increased despite the fall in cardiac output. These deviations from the expected relationship could be accounted for by the presence of spontaneous coronary dilatation which had been noticed before progressive heart failure had appeared (fig. 1) or by the increase of the systemic resistance which was made in an endeavor to keep aortic pressure constant. Both events would be expected to divert more of the cardiac output into the coronary bed. Another possible factor to be considered is that of an increase in the artificial systemic resistance, without measurable alteration of the pressure reading of the chamber surrounding the resistance tube, as the cardiac output fell. The reasons for this have been discussed previously (16). When spontaneous coronary dilatation continued in the presence of "forward" failure, the rate at which coronary flow rose lessened. When cardiac output markedly declined, eventually coronary flow no longer increased, or began to decrease.

Thus, all the changes in coronary flow seen during heart failure are attributable to the factors found to operate in non-failing hearts, viz., spontaneous coronary dilatation, changes in cardiac output and changes in the peripheral systemic resistance.

*d. Work of the heart.* Work, calculated as cardiac output multiplied by the sum of aortic and pulmonary arterial pressures, was found to vary with cardiac output, both in "congestive" heart failure and in "forward" failure. This is obviously due to the relatively stable level at which the arterial pressures stayed or were maintained. Only towards the end of "forward" failure was the work decreased out of proportion to the decline in cardiac output because at this time there was a decline in arterial pressures (fig. 1).

In calculating work of the heart in heart failure, one point deserves stress. Actually the work of the heart should not be measured by the level of the arterial pressures above atmospheric, but rather by the level above the pressures existing in the blood entering the respective ventricles. Ordinarily the changes in the venous pressures are so small as to introduce no serious error in the customary manner of calculating work. However, when failure occurs, these venous pressures rise considerably, and then the true work of the heart is to a varying

degree smaller than calculated (fig. 1). Thus, the work of the heart actually decreased in "combined" failure more and earlier than our calculations show, and, in "congestive" failure, as the venous pressure rose precipitously the actual work declined sharply even with a constant cardiac output. Thus, even in "congestive" failure, this decline in cardiac work introduces an element of "forward" failure even though arterial pressure and cardiac output be unchanged.

*Failure in heart-lung preparation.* Progressive heart failure was observed in 16 preparations<sup>4</sup>. The following is a breakdown of the forms of heart failure seen:

a. Right "congestive" failure.....	2
b. "Forward" failure.....	1
c. Combined left and right "congestive" failure.....	9
1, of which the congestion was dominant in the right.....	2
2, of equal degree in the two sides.....	7
d. "Mixed" form of heart failure.....	4
1, consisting of right "congestive" and "forward" failure.....	1
2, consisting of combined right and left "congestive" and "forward" failure (in all the congestive failure was of equal degree in the two sides).....	3

In these experiments, left "congestive" failure occurred in all but four instances, right "congestive" failure was absent in only one case. In only one instance was progressive heart failure present from the start, in the other 15 it developed in the course of the experiment.

In these 16 experiments the onset of failure began as:

a. Left "congestive" failure in.....	5
b. Right "congestive" failure in.....	6
c. "Forward" failure in.....	1
d. Left and right "congestive" failure in.....	3
e. Combination of all three forms in.....	1

Compared to the isolated heart, right "congestive" failure was more frequent at the start, and left "congestive" failure rarer in the heart-lung. As in the isolated heart, the duration of failure was shorter in those heart-lung preparations in which "forward" failure occurred in combination with "congestive" failure than when the latter occurred alone.

*Variations of the individual pressures and flows during progression of heart failure in the heart-lung.* The accelerating rise in venous pressures in "congestive" heart failure (figs. 3 and 4), the tendency for "forward" failure to stabilize the venous pressures (fig. 3) and the drop in right and/or left venous pressure when the aortic and/or pulmonary arterial pressure fell markedly, were also seen in the heart-lung preparation. However, left venous pressure rises were less frequent and less marked than the rise in right venous pressure in the heart-lung.

There is no particular difference in the alterations observed in cardiac output, cardiac work, and coronary and aortic flow between the isolated heart and heart-

<sup>4</sup> In 6 experiments the failure did not continue to completion because of accident, the introduction of a drug, etc.; in 4 of these, however, failure was severe when this happened.

lung preparations in the various types of heart failure, as figures 2, 3 and 4 will show.

The changes in aortic pressure in the heart-lung in the various forms of heart failure were similar to those in the isolated heart discussed earlier (see figs. 2, 3, 4). In "forward" failure, except in one instance, the fall in aortic pressure showed the same lag behind the fall in cardiac output as in the isolated heart; there was a delayed fall in two and no fall in two out of five instances of "forward" failure. In one instance aortic pressure began to fall before cardiac output declined, presumably due to coronary dilatation. This last occurred in the experiment in which progressive failure was present from the start.

The changes in pulmonary pressure, in contrast to the isolated heart, depended in part on the changes of left venous pressure and of the resistance of the lung vessels, whereas in the isolated heart the artificial separation of the pulmonary artery and the pulmonary veins made by means of the insertion of an open inflow reservoir connected to the left auricle prevents such dependence. Consequently, in the presence of "congestive" failure, pulmonary arterial pressure rose as left venous pressure rose (figs. 2, 3, 4 of this report and fig. 3 of a previous report (12)). Sometimes pulmonary arterial pressures rose more or began to rise earlier (fig. 4), or rose when left venous pressure did not; these findings indicate that the pulmonary vascular resistance had increased because of congestion and sometimes possibly because of pulmonary edema. The increased pulmonary vascular resistance in the presence of marked left congestive failure explains the stability of pulmonary arterial pressure in cases when "forward" failure complicated congestive failure. It also accounts for the relatively less marked changes in left venous pressure compared to the right venous pressure changes.

Thus, the changes in pulmonary arterial pressure in the heart-lung preparation are no different from those seen in the non-failing heart. The occurrence of pulmonary alveolar wall edema, increasing the pulmonary vascular resistance is however almost pathognomonic of heart failure. In this respect, the heart-lung preparation is more informative than the isolated heart of the changes in congestive heart failure in the intact circulation. In man, the accentuated pulmonic second heart sound, often heard in heart failure is evidence that pulmonary arterial hypertension does occur clinically, since it has been shown that the intensity of the second sound is a function of the height of the arterial pressure beyond the semilunar valves, here the pulmonary artery, at the time when these valves close (17). The causes of the pulmonary arterial hypertension probably include those described in the heart-lung preparation.

*Effect of increase in load on the heart on the degree of heart failure.* On several occasions, increasing the cardiac output or the resistances in the systemic and/or pulmonary circuits brought about or accentuated heart failure which sometimes disappeared or decreased when the load was again reduced. There occurred an unexpectedly great elevation of the venous pressure(s) beyond that seen in non-failing hearts on similar increase of load. Similarly, increasing the volume of blood in circulation when the heart was in severe failure sometimes increased the venous pressure(s) without increasing cardiac output, and this

change could often be reversed by withdrawing blood. These observations have several important implications: 1, they show that in a heart capable of dealing with a small load without development of failure, failure may be precipitated by an increased load; 2, they suggest that the failing heart shows greater degrees of congestion (and distention) when its load is increased than the non-failing heart, thus lending direct support for the view that reduction of load has an immediate effect in alleviating heart failure and conversely, that increasing the load on the failing heart actually aggravates the degree of failure and presumably accelerates its downhill course.

#### SUMMARY

In the special heart preparations discussed in previous communications, cardiac failure was studied in 22 isolated heart and 16 heart-lung experiments. The reasons for development of failure when the heart is removed from the body are unknown; failure eventually terminates the experiment. The manifestations of failure are 1, increase of one or both venous pressures without increase in cardiac output, and/or 2; decrease of cardiac output (pulmonary flow) and often also aortic pressure (the latter usually later if at all). Once initiated, cardiac failure pursues an apparently vicious accelerating course with the slopes becoming progressively steeper in their upward or downward trends, and especially when aortic pressure begins to fall the failure accelerates tremendously. All types of failure in our experiments are due to decreased power of the heart, but where the cardiac output and arterial pressures are level and the venous pressure rises on the left and/or right side, left and/or right "congestive" failure is present. In the presence of one or both types of failure the increased venous (i.e., auricular) pressures with increased diastolic volume, enable the work to be maintained. When the venous pressures do not rise, but work of the heart (cardiac output and/or arterial pressures) decreases, "forward" failure is present. Failure thus may be of the left or right "congestive" type, of the "forward" type, of combined left and right "congestive" type, or of "mixed" (congestive and forward) type. In general, the steepness of the slopes of the curves referred to is an expression of the degree of severity of the failure present.

*Isolated heart preparation.* Aortic pressure fell in only half the cases of "forward" failure, and in more than half of those in which it fell, the decline in aortic pressure was preceded by a decline in cardiac output; in three experiments both began to fall at the same time. Delayed or absent fall in aortic pressure is due in some to decline in coronary flow with the consequent increase in coronary resistance being sufficient to maintain the total resistance of the two parallel circuits through which the left heart output is distributed. The usual cause, however, for the maintenance of aortic pressure is the existence of a critical level of aortic pressure and cardiac output above which changes of the latter do not appreciably affect the former. This is due to the peculiar relationship of these two in the existent distensible circuit discussed in the text and more fully in a previous report (15). This fall in arterial pressure is a sign of advanced, rather than early failure. In congestive failure per se, aortic pressure remained constant. Pulmonary pressure relationships are similar to those of aortic pressure,

except that coronary resistance increase cannot help maintain pulmonary pressure. In left congestive failure, the equivalent of a gap in the circuit in the isolated heart preparations was responsible for the lack of rise of pulmonary arterial pressure.

With left or right "congestive" failure, left or right venous pressure rose in an accelerating fashion. The development of "forward" failure lessens the rate of rise or even produces a fall—distorting the otherwise exponential type of curve. The venous pressures are increased by the congestion consequent upon decreased power of the heart but are decreased by the lessened load of the heart when the work declines in "forward" failure. Regurgitation due to dilatation of the atrio-ventricular valve rings in advanced failure may contribute to the rise of venous pressures. Decreased cardiac tonus in failure may lessen this rise.

Coronary flow changes in "congestive" failure, that is, changes in the partition of the cardiac output, occur as in non-failing hearts. In "forward" failure coronary flow usually decreases when cardiac output falls. Delay or absence of decrease was due, in our experiments, to 1, continuance of spontaneous coronary dilatation present before "forward" failure; 2, increase in peripheral resistance due to adjustment to keep aortic pressure from falling, and 3, increase in peripheral resistance due to decreased distention of the resistance tubing as the aortic-vena cava flow decreases. Thus no changes in coronary flow occur which are peculiar to heart failure per se.

Calculated work of the heart was found to be similar to cardiac output except when marked "forward" failure was associated with a drop in arterial pressures. However, severe "congestive" failure significantly lessens the true work of the heart since with pressure already high in the blood as it returns to the heart, less work is necessary to raise the pressure to the same degree of arterial pressure. This has been neglected in ordinary calculations of work, which have hitherto ignored initial pressures of the entering blood.

*Closed-circuit heart-lung preparation.* Apart from the fact that in these experiments artificial maintenance of constant cardiac work (when possible) delayed or prevented "forward" failure, changes were essentially similar to those occurring in the isolated heart, except with regard to pulmonary arterial pressure and left venous pressure. Due to the absence of artificial separation of the pulmonary artery and "pulmonary veins," pulmonary arterial pressure rose in left congestive failure. Moreover, pulmonary edema, when present, caused an accentuated rise in pulmonary arterial pressure and lessened the left venous pressure rise. Both factors lessened the tendency of pulmonary arterial pressure to fall in some cases when "forward" failure developed. In the isolated heart, a similar difference was seen between those experiments in which cardiac output was maintained constant and in those in which it was not.

*Effect of increase in load.* On several occasions, increasing the cardiac output or artificial resistance initiated or accentuated failure as manifested by the changes in venous pressure. Similarly, in severe failure increasing the blood volume sometimes increased the venous pressures but not the cardiac output, and withdrawing blood sometimes reversed this effect.



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# PROXIMO-DISTAL FLUID CONVECTION IN THE ENDONEURIAL SPACES OF PERIPHERAL NERVES, DEMONSTRATED BY COLORED AND RADIOACTIVE (ISOTOPE) TRACERS<sup>1</sup>

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Peripheral nerves contain four different kinds of channels in which substances may be transported: the blood vessels, the lymphatics of the sheath, the axons, and the spaces between the axons which we shall call the endoneurial spaces. The vascular supply of nerves has been dealt with in excellent review papers of recent date (Adams, 1942, 1943; Bentley and Schlapp, 1943), and the more intricate problem of intra-axonal and inter-axonal traffic has been very competently discussed in the monograph of Howe and Bodian (1942), according to which toxins and neurotropic viruses seem to spread preferentially inside the axis cylinders, taking the ascending direction (see also Speransky, 1935).

Observations of edema formation in constricted peripheral nerves, on the other hand, intimated that fluid outside and between the nerve fibers moves in the descending direction (Weiss, 1943a; Weiss and Davis, 1943).

The centrifugal direction of this postulated endoneurial seepage was, however, at variance with earlier reports according to which the direction of fluid convection in peripheral nerves is mostly towards the spinal cord (e.g., Teale and Embleton, 1914, 1919; Yuien, 1928). Those earlier claims were based on observations on the spread of various substances, including bacterial suspensions injected into large nerves. However, the excessive amounts of fluid used in the injections (e.g., 0.2–0.5 cc. of fluid into a rabbit sciatic) must have created such unnatural hydrostatic pressure conditions in the nerves that the natural physiological flow might have been fully obscured by this artifact.

In view of the conflicting and inconclusive state of the problem, the series of experiments here described was undertaken. Marker substances used were India ink, Chinese ink, potassium ferrocyanide (Weed, 1917) with subsequent Prussian Blue precipitation, and radioactive tracer substances.

The results obtained on a total of 420 nerves prove that in the limb nerves of rats and guinea pigs, endoneurial transport is directed toward the periphery, and not toward the cord.

All experiments were done with the sciatic nerve or its two major divisions or with brachial nerves of albino rats and guinea pigs. As rat nerves are poorly

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fasciculated, any injection into the trunks leads directly into the endoneurial spaces. All injections in situ were performed under nembutal anesthesia. The nerves were exposed, and all adhering liquid was blotted off.

I. *India ink tests.* Injection of cca. 0.1 cu. mm. of India or Chinese ink suspension into 46 nerves in situ showed in the majority of cases greater spread of ink particles in the distal than in the proximal direction during the first several hours after the operation. Clumping of the granules and their adhesiveness to the tissue, however, led to early clogging of the endoneurial channels, and the method was abandoned as inadequate.

II. *Prussian blue tests. Method.* Convection of colloidal and particulate dyes in nerves has been studied previously (Teale and Embleton, 1919; Yuien, 1928; Uljanov, 1929; Yuien and Sato, 1929; Perdrau, 1937), but since our experiences with ink clumping contraindicated the use of particulate matter, we turned to the methods of Weed (1917) and Wishnewsky (1928) using potassium ferrocyanide in solution followed by Prussian Blue precipitation.  $K_4Fe(CN)_6$  is introduced into the nerve, allowed to diffuse for varying periods of time, and then the nerve is placed into a 10 per cent solution of ferric chloride, which precipitates Prussian Blue. Bathing the nerve for two minutes in  $H_2O_2$  before immersing in  $FeCl_3$  intensifies the color of the Prussian Blue reaction.

In the first group of experiments the ferrocyanide was injected in solution. Pipettes tapering to 0.2 mm. width at the mouth or minute cotton wicks inserted through a lateral puncture were used to introduce the solution. Approximately 0.1 cu. mm. of the substance was injected by slight pressure. A drop of paraffin oil was used to seal the wound and prevent escape of the injected mass. The injected amount was so small that in no case was there any perceptible disruption of the nerve, except a slight herniation of fibers through the hole in the sheath after withdrawing the pipette.

In order to eliminate all injection pressure, we finally turned to introducing  $K_4Fe(CN)_6$  in crystalline form. The sheath was punctured with a glass needle, and a minute flake of the salt was placed lengthwise between the nerve fibers without injury to the latter. The hole was sealed with paraffin oil. The crystal became completely dissolved within 5 to 10 minutes.

All experiments were done with rat hind-limb nerves in situ, either intact or severed, some in dead animals for controls. After from one half to nine hours, the nerves were excised with special care to avoid displacement of the endoneurial liquid.

The injection point was always clearly discernible. After some practice, it was easy to tell where the stained zone ended, and checks by different observers on the same preparations never disagreed by more than one millimeter. Microscopic examination proved the location of the stain to be exclusively endoneurial, i.e., between the nerve fibers; the substance had not penetrated into the axons themselves.

The anatomical uniformity of the nerve stretches used for these tests has been confirmed by diffusion tests in nerves injected several hours after the death of an animal. Table 1 shows the progress of longitudinal diffusion in such nerves:

there is no significant asymmetry between the ascending and descending directions.

*A. Fluid injections.* In contrast to the results with dead nerves, fluid injected into live nerves in situ assumed in most cases an asymmetrical distribution, extending farther in the distal than in the proximal direction. Cases with fluid injection, no matter how performed, are lumped in the following compilation.

Out of a total of 77 cases, fourteen (i.e., 18 per cent) showed symmetrical spread in both directions; fifty-five (i.e., 72 per cent) greater spread in the distal direction, the excess averaging 4.6 mm.; while only eight (i.e., 10 per cent) showed greater spread in the proximal direction, with an average difference of 2.2 mm. Or, if cases with no more than 1 mm. asymmetry are counted as symmetrical, the figures are: symmetrical, 23 (30 per cent); greater distal spread,

TABLE 1  
*Diffusion of  $K_4Fe(CN)_6$  in nerves of dead animals*

NERVE	DURATION OF EXPERIMENT	EXTENT OF DIFFUSION			ASYMMETRY (D-P)
		proximal (P)	distal (D)	total (P + D)	
	<i>hrs.</i>	<i>mm.</i>	<i>mm.</i>	<i>mm.</i>	<i>mm.</i>
Tib.....	1	3	3	6	0
Per.....	1	3	3	6	0
Tib.....	1½	3.5	3.5	7	0
Per.....	1½	3.5	3.5	7	0
Tib.....	5	6.5	6.5	13	0
Per.....	5	6.5	6.5	13	0
Tib.....	7	8	9	17	1
Per.....	7	8	9	17	1
Tib.*.....	1	9	9	18	0
Tib.*.....	5	13	13	26	0

\* Nerves with implants of crystals instead of injections.

78 (62 per cent), with an average difference of 5.1 mm.; greater proximal spread, 6 (8 per cent) with an average difference of 2.7 mm.

There is thus a decidedly greater spread of substance in the descending than in the ascending direction. This effect appears no matter whether the injection pipette is pointed proximal or distal. It is less marked after injections in perpendicular direction, presumably because this operation involves practically the whole width of the nerve.

Figure 1 gives a graphic summary of the results arranged according to time. For the reason just mentioned, the cases with transversal injection have been excluded. Of the 29 cases with ascending or descending injection, all but two showed a positive distal differential. While the stained area has reached its full extent as early as one half hour after injection, the stain has shifted progressively in the distal direction, as can be seen from the increasing distance between the center of the stained area (white circle), and the injection point (dark circle). This shift is most marked during the first half hour, then declines rapidly, and

seems to have ceased during the second hour. This indicates that the injected substance produces changes in the tissue which gradually obliterate the channels, or otherwise interfere with the mechanism, of convection.

Separate computations for the 23 peroneal and 32 tibial nerves show a greater average distal excess in the former (5.1 mm.) than in the latter (4.1 mm.), a fact which may be correlated with the size difference between these nerves. Even in the India ink experiments it was noted that the distal shift tends to be more marked in smaller nerves.

*B. Crystal injections.* In 51 experiments, crystals of  $K_4Fe(CN)_6$  were introduced into the nerves. This method eliminates the variables of direction and pressure of injection. Accordingly, results were more uniform than in the preceding series. Of the 51 cases, four (8 per cent) showed equal spread in both directions; forty-three (84 per cent) greater spread distad, exceeding the proximal spread by an average of 5.4 mm., while only four (8 per cent) showed proximal excess, averaging 2.3 mm.

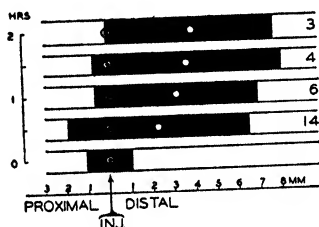


Fig. 1

Fig. 1. Distribution of  $K_4Fe(CN)_6$  injected in solution into live nerve in situ. Number of cases in each class given at right margin.

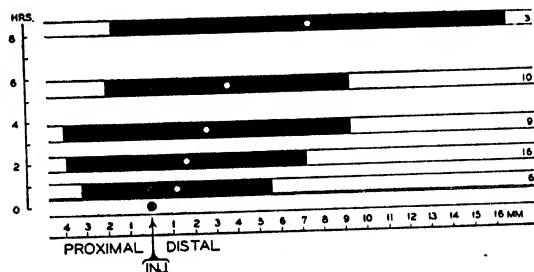


Fig. 2

Fig. 2. Distribution of  $K_4Fe(CN)_6$  in nerve in situ after introduction in crystalline form. Numbers at right margin indicate number of nerves in each class.

The time course of the distal shift is illustrated in figure 2. The graph was obtained by breaking the 43 positive cases up into 5 groups according to time, as indicated in the figure. Just as in the fluid injection tests, the diffusion field does not continue to expand appreciably after the first or second hour. The shift of the whole field in distal direction, however, may continue for several hours, as evidenced by the progressive increase with time of the distance between the center of the stained zone (white circle) and the injection point (black circle). Unlike the preceding series A, convection had not come to a stop even after six hours. (The disproportionately great shift in the 7½-9 hr. group cannot be considered as typical because of the small number of cases.) Later the stain begins to fade; five cases examined 16 hours after injection showed no more stain left.

This series thus confirms the existence of proximo-distal fluid convection in live nerve, particularly if series A and B, based on a total of 128 cases, of which 98 (i.e., 77 per cent) were positive, are taken in conjunction.

*C. Experiments with cut nerves.* Nerves were first treated as in the preceding series, but after injection were severed at levels either proximal or distal to the site of injection, or both. The cuts were placed far enough from the point of injection to allow adequate space for diffusion. The results of 53 experiments reveal the following facts. The total length of the diffusion field is of the same order as in the intact nerves of series A and B; it is longer after implantation of a crystal than after fluid injection. The distal asymmetry, however, has failed to develop except in two series, comprising ten cases, in which the injection had been pointed in the distal direction (average distal excess, 3.5 mm.). Evidently, nerves still contain the mechanism of proximo-distal convection even after transection. But the operation of this mechanism was in most cases suppressed by cutting the nerve. The location of the cut did not seem to matter, and a single cut was as effective as complete isolation.

III. *Tests with radioactive tracer substances. Method.* By the use of radioactive isotopes, the diffusion field can be plotted out quantitatively. For practical purposes, the radioactivity of any given segment of the injected nerve is a direct function of the concentration of the injected substance in that segment, provided allowance is made for the natural decay of radioactivity. Our choice of appropriate substances was determined primarily by considerations of experimental expediency. Some of the selected substances were highly toxic, but since only short-term experiments were contemplated, this fact was of no consequence.

Three substances were used:  $\text{Na}^{24}\text{Cl}$  (half-life of 14.8 hrs.),  $\text{Cu}^{64}\text{Cl}_2$  (half-life of 12.8 hrs.), and practically insoluble  $\text{Cu}^{64}\text{S}$  (half-life of 12.8 hrs.).

The salts were deposited in the nerve as solid pellets made by first pulverizing the crystals and then compressing the powder in the finely drawn out tip of a glass pipette (0.15 to 0.3 mm. wide). A glass thread fitting the bore of the pipette served as plunger to force the pellet out, after the mouth of the pipette had been thrust through the sheath and eased into the interior of the nerve. Each pellet was from 1 to 2 mm. long, corresponding to cca. 0.1 to 0.4 mgm. of substance. The direction of injection was alternately up and down, so that for a given lot of nerves any possible asymmetry from this source cancels out. Even without this precaution, errors due to the direction of injection are highly improbable, since the mass is introduced in the solid state. The nerve was superficially dry at the time of injection, and special care was taken to avoid the escape of substance to the surface. The sodium chloride and copper chloride powders dissolved promptly.

The experiments were done either *in situ*, with or without additional interventions (nerve transection, ligation of blood supply), or *in vitro*. In the former case, the wound was closed and the animals were allowed to recover from nembutal anesthesia. In the latter case, the nerves were excised and kept in moist chambers at  $37^\circ\text{C}$ ., stretched out horizontally along silk threads, glass rods, or on paraffin-coated glass plates. *In vitro* experiments required particular caution to prevent surface contamination by capillary spread between nerve and supports.

After a lapse of from  $1\frac{1}{2}$  to 48 hours, the injected nerves were dissected, if in

situ, or removed from the moist chambers, if in vitro. From there on, both groups were treated alike. All nerves were first dried on glass plates, then cut into smaller segments of equal length. The injection site, always clearly discernible, served as landmark. A piece of nerve measuring 5 mm. to either side of this point was excised; it included the original injected zone plus a safe margin. This piece will be designated as "center piece" or "O." Moving from it in both directions, the rest of the nerve was then cut with a clean sharp blade into 5 mm. fragments (10 mm. in the early series), consecutively labelled as  $P_1, P_2, P_3 \dots$  and  $D_1, D_2, D_3 \dots$  for the proximal and distal direction, respectively. Thus, identical index (e.g.,  $P_2$  and  $D_2$ ) indicates conjugated samples located symmetrically and equidistant from the injection point.

Because of the small sizes involved, several nerves were used for each experiment. After cutting, all fragments from the same levels were lumped in dry depression slides: one slide received all  $P_1$  fragments, another all  $D_1$ s, a third the  $P_2$ s, etc. Owing to the unequal length of the different nerves, the terminal segments were often fewer in number; allowance for this fact was then made in the calculation of the average radiation per unit.

Blood and tissue samples from treated and untreated control animals, as well as solutions to be tested for radioactive contents, were evaporated in depression slides and then assayed in the dry state.

Radioactivity was measured with a Geiger-Müller counter equipped with an automatic recording device. Each depression slide, containing the lumped samples from the same level of all nerves used in the particular experiment, was exposed separately. Position and distance from the counting tube were rigorously kept constant. The counting period was usually 5 minutes. In order to make corrections for radioactive decay unnecessary, conjugated pairs were always counted in immediate succession. Thus the order of counting was mostly  $O-P_1-D_1-P_2-D_2-P_3-D_3-P_4-D_4$ , etc. These nerve segments are strictly comparable only within the stretch over which the size and structure of the nerve remain unchanged. This was true of all but the most remote levels (6 and 7), as  $D_6$  and  $D_7$  lacked a few small peripheral branches leaving the common trunks at  $D_5$ . Counts at the extreme levels therefore favor the proximal segments.

The "background" of stray radiation in the room was determined before and after the nerve samples were exposed and at 20 to 30 minute intervals in between. The mean of 147 background recordings over a four months period was  $21.95 \pm 0.39$  quanta/min. ( $\sigma = 1.74 \pm 0.28$ ). This however includes a two weeks' period during which the counter went out of order and registered 2-3 quanta per minute above average. Outside of this period the maximum fluctuation observed during any one day was  $\pm 3$  quanta. All values quoted in the tables express actual radiation from the specimens, i.e., total radiation minus the average of background readings taken over the course of the given experiment.

In the following description, experiments in rats and guinea pigs are treated jointly; they are designated by the letters "R" and "GP," respectively. In the graphs, the abscissa represents the axis of the nerve, with the assayed segments

arranged serially in proximo-distal order. The ordinates give the counter readings per minute for each level. These readings are the sum of the background radiation and active radiation from the specimen. Background radiation is recorded in the graphs as a band between the maximum and minimum background readings obtained in the course of the experiment. All values above this band express radiation actually issuing from the sample; they are represented as bars. The varying height of these bars reflects the distribution (concentration) of the substance in the nerve. Differences of concentration between segments located symmetrically with respect to the injection site are represented in the graphs by the black portions of the higher columns.

The following report is based on a total of 35 experiments (23 *in situ*, 12 *in vitro*) comprising 193 nerves.

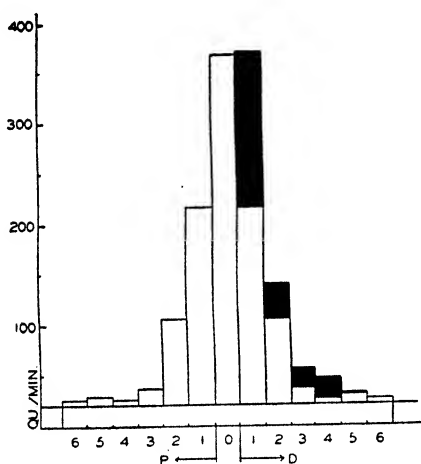


Fig. 3

Fig. 3. Distribution of radioactive  $\text{Na}^{24}\text{Cl}$  in 10 rat nerves (R6-10), 3 hours after injection.

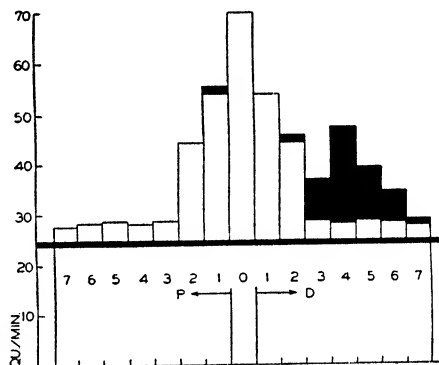


Fig. 4

Fig. 4. Distribution of radioactive  $\text{Na}^{24}\text{Cl}$  in 6 rat nerves (R15-17), 18 hours after injection.

*A. Injections of  $\text{Na}^{24}\text{Cl}$ .* This group consists of 7 experiments with 33 nerves *in situ*, and one experiment in which the nerves had been cut and which will be discussed in a later chapter. The results of two typical experiments are reproduced in the graphs, figures 3 and 4.

Experiment R6-10 (fig. 3), in which the left and right sciatic nerves of five rats had been injected was terminated after 3 hours. At this time the substance had diffused up and down the nerve and assumed the distribution shown in the graph. It can readily be seen that concentrations are consistently higher at the distal levels than at the corresponding proximal levels up to and including level 4 (25 mm. from injection point). More substance has spread in the distal than in the proximal direction.

Experiment R15-17 (fig. 4) in which the right and left sciatic nerves of 3 rats



had been injected—with the pellet in all six cases being deposited proximal to the puncture—was continued for 18 hours. The distribution of radioactive substance in the nerve after this period reveals the following facts:

1. The concentration is still highest at the original injection site, and falls off rather sharply to either side. Since sodium chloride with its high solubility must have long since diffused out from the region, it is obvious that some of the radioactive sodium had become fixed in less soluble form in the tissue, thus preserving some of the shape of the original concentration gradient.

2. There is no significant difference between the proximal and distal fragments at levels 1 and 2.

3. The distal segments 3, 4, 5 and 6 contain radioactive sodium greatly in excess of the amount present at the corresponding proximal levels. The low intensity and relative constancy of the radiation from  $P_3$  through  $P_7$  suggest that these parts of the nerve have obtained their  $Na^{24}$  from the blood and not by

TABLE 2  
*Distribution of  $Na^{24}Cl$  injected into nerves in situ*

EXP.	NO. OF NERVES	DURATION OF EXP.	CENTER PIECE O	$\Sigma P$ QUANTA/MIN.	$\Sigma D$ QUANTA/MIN.	DISTAL EXCESS D-P QUANTA/MIN.	ASYMMETRY (D > P)
		<i>hrs.</i>					
R 1 L.....	1	3½		64	146	82	+
R 2-4.....	4	3		2936	3318	382	+
R 3.....	2	2½		584	854	270	+
R 5.....	2	24	30	4	14	10	+
R 6-10.....	10	3	347	320	546	226	+
R 11-14.....	7	5	75	107	148	41	+
R 15-17.....	6	18	46	87	123	46	+

direct diffusion from the center piece. Distally, this condition is not reached until  $D_7$ . Only the zone from  $P_2$  to  $D_6$  contains concentrations that can be ascribed to direct diffusion. As the center of this area lies in  $D_2$ , it is evident that the whole diffusion field has shifted distad by at least as much as the distance between levels O and  $D_2$ , i.e., 10 mm. This value does not express the true rate of the shift, because, as stated in point (1), after 18 hours we are no longer plotting the original diffusion field but only its fixed residue.

The remaining five experiments gave essentially similar results. They are summarized in table 2. This summary treatment ignores the distribution gradients and merely compares the total amount of radioactive substance found distally to the injection point with that contained in the corresponding proximal stretch of nerve. Radiation intensities varied greatly from experiment to experiment, owing to differences in the initial potency and the degree of decay of the different radioactive preparations at the times they were used. The very low count of R5 is explained by the long duration of the experiment (24 hrs.), allowing for the washing out and excretion of the injected substance. Otherwise the results are uniform. All tests show a distinct excess of substance in the distal portions, rising to as much as several hundred quanta per minute.

*B. Injections of  $\text{Cu}^{64}\text{S}$ .* Four experiments with 19 nerves were carried out in this series. In line with the low solubility of the salt, no radiation was detected in any nerve segments except the ones containing the original injected mass. Only minute traces could be detected in the blood of these animals. Radiation intensities in the center pieces of the four groups were 19232, 9644, 1583 and 1726 quanta per minute after 6, 25, 42 and 48 hours, respectively. The contrast between these high values and the absence of activity in even the next adjacent segments not only proves the complete lack of diffusion in these cases, but provides a crucial test for the general reliability of the technique, since any traces of contamination due to handling would have produced positive counts.

*C. Injections of  $\text{Cu}^{64}\text{Cl}_2$ .* This substance, which owing to its bluish tint could easily be recognized after its deposition in the nerve, did not dwindle as rapidly

TABLE 3

*Differential distribution of radioactivated salt in nerve segments nearest to injection site*

EXPERIMENT	NO. OF NERVES	DURATION OF EXP.	INJECTED PIECE (O) QUANTA/MIN.	$P_1$	$D_1$	DISTAL DIFFERENTIAL		ASYMMETRY $D > P \dots +$ $D = P \dots =$ $D < P \dots -$
						$D_1 - P_1$ quanta/min.	Percentage	
		<i>hrs.</i>					<i>per cent</i>	
GP 1-2...	3	4	2040	221	289	68	31	+
GP 6-7...	4	5	6144	689	829	140	20	+
GP 12-13...	4	6	7099	829	708	-121	17	-
GP 4-5...	4	18	544	17	25	8	insign.	=
R 32-40....	10	4	5760	1464	458	-1006	220	-
R 41-43....	4	16	3908	107	109	2	insign.	=
Total....	29		25495	3327	2418	-909	38%	-

as did  $\text{Na}^{24}\text{Cl}$ . Consequently, it was still visible at the end of an experiment as a distinct blue spot. Since the radiation intensities of the center piece and the adjacent  $P_1$  and  $D_1$  segments were too high to be shown on the same scale with the much weaker radiations of the more distant levels 2, 3, 4, etc, they were omitted from the graphs and are given in tabular form later (table 3).

There were 6 experiments comprising 29 nerves in which  $\text{Cu}^{64}\text{Cl}_2$  was injected into nerves in situ with no further interference. Two sample cases are shown in the graphs (figs. 5 and 6), both of 4 hours' duration, the first with 3 nerves in guinea pigs, the second with 10 nerves in rats. The longer nerves of guinea pigs yield more 5 mm. segments for testing, and being more fasciculated than rat nerves, they offer interfascicular spaces for liquid transfer. Yet, there were no essential differences between the two groups. Both show a spectacular excess of radioactivity in the distal segments over the corresponding proximal ones. In experiment GP 1-2 (fig. 5), the presence of directly diffused substance (i.e., not carried through the blood) is demonstrable between levels  $P_5$  and  $D_{10}$ . It presumably extended beyond  $D_{10}$ , where nerve samples were not assayable for

anatomical reasons. The geometric center of the radioactive area, accordingly, lies between  $D_2$  and  $D_3$ , which corresponds to a total shift of the diffusion field down the nerve of cca. 15 mm. in 4 hours. In experiment R32-40 (fig. 6), the distal spread has likewise gone beyond the limits of the nerve stretch dissected for assaying, but by extrapolation from the graph it may be estimated to have extended into  $D_8$ , which would bring the approximate center to  $D_2$ , corresponding to a distal shift of cca. 10 mm. in 4 hours.

Of the remaining 4 experiments, done with 16 nerves and ranging from 5 to 18 hours, the 18-hour one (G.P. 4-5) gave very low counts (compare table 3) with a correspondingly small, though definite, distal overbalance ( $\Sigma D = 70$ ;  $\Sigma P = 46$ ). Evidently, most of the substance had disappeared by this time. The results of the other three experiments (GP 6-7, 5 hr.; GP 12-13, 6 hr.;

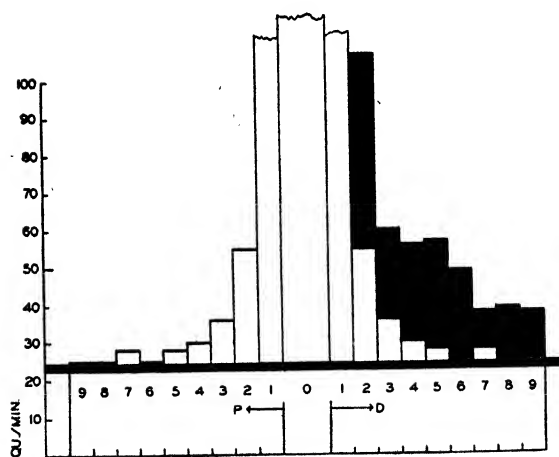


Fig. 5

Fig. 5. Distribution of radioactive  $\text{Cu}^{64}\text{Cl}_2$  in 3 guinea pig nerves (GP 1-2), 4 hours after injection.

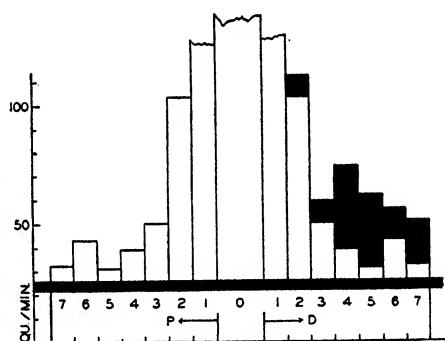


Fig. 6

Fig. 6. Distribution of radioactive  $\text{Cu}^{64}\text{Cl}_2$  in 10 rat nerves (R32-40), 4 hours after injection.

R 41-43, 16 hr.), all essentially alike, have been lumped into a single graph, figure 7. The marked distal shift of the diffusion field for the whole lot is evident.

In contrast to these fully consistent results with segments from levels 2 and beyond, the counts from segments  $P_1$  and  $D_1$  were erratic. They are given in table 3. There was no significant difference between  $P_1$  and  $D_1$  in some cases; others showed a positive distal, and still others a positive proximal differential. A discussion of this fact will be given later.

*D. Injections combined with other interventions.* The following experiments constitute an attempt to elucidate the mechanism of the proximo-distal convection effect.

*Stoppage of blood circulation.* On a previous occasion (Weiss, 1943a), the possibility was discussed that endoneurial fluid might be propelled by the

arterial pulse. In order to test this hypothesis, the right and left sciatic nerves of two guinea pigs were injected with  $\text{Cu}^{64}\text{Cl}_2$  as usual, and then circulation was stopped by ligating the aortae near the heart. The nerves were left in situ for 5 hours and then dissected and assayed. The results are shown in figure 8. Evidently, a shift of the diffusion field distad had occurred. This result disproves the assumption that the pulse wave, or any other circulatory factor, furnishes the driving force for the endoneurial shift of fluid. It also shows that death of the animal does not immediately stop the shift. According to table 1, however, the shift no longer occurs a few hours after death.

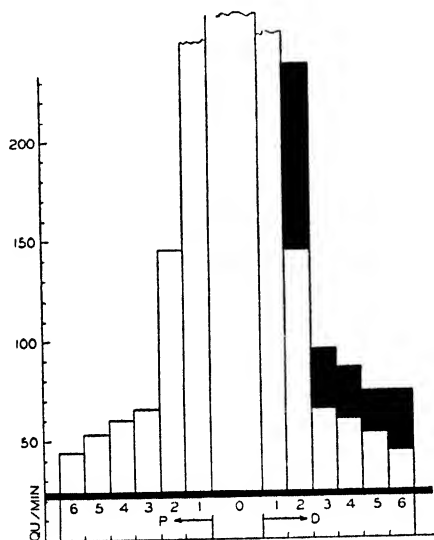


Fig. 7

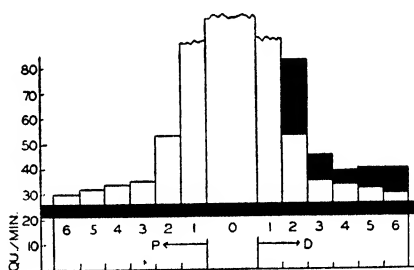


Fig. 8

Fig. 7. Distribution of radioactive  $\text{Cu}^{64}\text{Cl}_2$  in 12 nerves, 6-16 hours after injection.

Fig. 8. Distribution of radioactive  $\text{Cu}^{64}\text{Cl}_2$  in 4 nerves of guinea pigs with arrested circulation, 5 hours after injection.

*Injection of transected nerves.* In this series, 16 sciatic nerves in 4 groups were first transected far proximally and distally and then injected about half way between the cuts with  $\text{Cu}^{64}\text{Cl}_2$ , but otherwise left undisturbed. Assays after 4 to 6 hours gave the following results. One animal (GP 11) had died shortly after the injection and 6 hours later showed no significant asymmetry of the diffusion field within levels 2, 3, 4 (transections in  $P_5$  and  $D_5$ ). Two series (8 nerves) showed a definite distal shift (table 4) and one series (6 nerves) showed a distal surplus at levels 3 and 4, but a distal deficit at level 2. (For counts of segments  $P_1$  and  $D_1$ , see table 5.) The results prove that a distal shift may still occur after the continuity between the injected stretch and the rest of the nerve has been interrupted. However, the absence of the effect in one case and its partial reversal in another are perhaps significant in view of a similar variability noted in transected nerves tested with Prussian Blue (series II C), as well as in the completely excised nerves described in the following.

*Injection of excised nerves in vitro.* This series includes 6 experiments with 52 nerves, injected with  $\text{Cu}^{64}\text{Cl}_2$  in vitro and then incubated in moist chambers for from  $1\frac{1}{2}$  to 5 hours. The results were not consistent. In the shortest experiment (4 rat nerves;  $1\frac{1}{2}$ –2 hr.), there was no significant asymmetry (R27–30; table 5), conceivably because of insufficient time. Another experiment (R1–10; 10 nerves, 4 hr.) showed a proximal surplus from  $P_1$  through  $P_4$ , but in this set the nerves had been hung across horizontal threads in chain-like fashion and the alternation between sagging and supported parts may have affected diffusion.

TABLE 4

*Distribution of  $\text{Cu}^{64}\text{Cl}_2$  injected into transected nerves in situ (GP 10, 2 nerves, 6 hours; R44–46, 6 nerves, 4 hours)*

LEVEL	2	3	4	5	6
D	488	45	27	21	14
P	58	24	14	11	10
D – P	430	21	13	10	4

TABLE 5

*Concentration of  $\text{Cu}^{64}\text{Cl}_2$  in the center piece and adjacent segments  $P_1$  and  $D_1$  of transected nerves in situ or in vitro*

EXPERIMENT	CONDITION	NO. OF NERVES	DURATION	CENTER PIECE (O) QUANTA/MIN.	$P_1$	$D_1$	ASYMMETRY		ASYM-METRY IN REST OF NERVE BEYOND $P_1$ AND $D_1$
							Sense	Amount	
GP 10.....	in situ	2	6	9963	808	236	D < P	572	D > P
R 37–39.....	in situ	6	$4\frac{1}{2}$	2443	605	530	D < P	75	D > P
R 44–46.....	in situ	6	4	21484	2302	1998	D < P	304	D > P
GP 15–26.....	in vitro	12	$3\frac{1}{2}$	56449	2015	1585	D < P	430	D > P
R 1–10.....	in vitro	10	4	14812	615	111	D < P	504	D < P
R 11–18.....	in vitro	8	4	90604	9577	2892	D < P	6685	D > P
R 19–26.....	in vitro	8	$4\frac{1}{2}$	20012	182	257	D > P	75	D > P
R 27–30.....	in vitro	4	$1\frac{1}{2}$	5432	11	13	D = P	2	D = P
R 55–64.....	in vitro	10	5	17045	4942	1721	D < P	3221	D < P

A third experiment (R55–64; 10 nerves, 5 hr.) showed a marked proximal surplus in  $P_2$  and  $P_3$ , and a very slight distal surplus in  $D_4$  and  $D_5$ . The remaining three experiments (12 guinea-pig nerves,  $3\frac{1}{2}$  hr.; 16 rat nerves;  $4$ – $4\frac{1}{2}$  hr.) produced a very pronounced distal shift (fig. 9). In one of these experiments (R11–18; table 5), a very potent salt preparation was used, and this accounts for the unusually high values in this group.

Taking these results in conjunction with those of the preceding section and those of section II C, two facts emerge. First, proximo-distal convection is still demonstrable in completely isolated nerve fragments, and second, this shift may in certain cases be abolished or even wholly or partly reversed as a

result of transection. Whether the transected nerves are left in situ or are transferred into moist chambers of body temperature, seems to have no influence on the results.

The counts at levels  $P_1$  and  $D_1$  adjoining the center piece are given in table 5. In 7 out of 9 experiments with transected nerves in situ and in vitro, the concentration of the diffused substance was lower in  $D_1$  than in  $P_1$ . A careful check of our procedures has convinced us that this paradoxical relation is to be ascribed to a slight, but systematical, error in the cutting of the nerve samples under the binocular microscope. The coincidence between the injection point and the null point of the measuring scale was established by binocular vision, while the

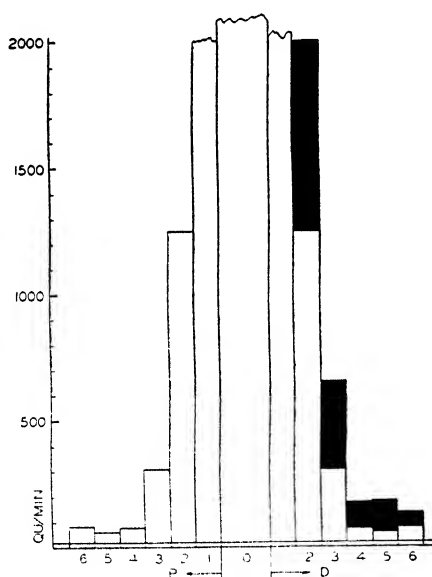


Fig. 9. Distribution of radioactive  $\text{Cu}^{64}\text{Cl}_2$  in 28 excised nerves,  $3\frac{1}{2}$ – $4\frac{1}{2}$  hours after injection.

5 mm. marks were viewed with the right eye only. This produced a parallax shift of 0.5 mm. to the right from the injection point, enough to give the observed asymmetry in the center piece, where the concentration gradient is very steep. This error is negligible for the more distant segments with gentler concentration slopes.

*Nerve ends in pools.* This series was designed to test possible polarity in the leaking of substance from cut nerve ends and in the diffusion into a nerve through its cut surface. The experiments could not be carried beyond the exploratory state and deserve repetition on a larger scale.

Three experiments with 14 nerves were set up to examine polarity of leakage. The nerves were excised and injected with  $\text{Cu}^{64}\text{Cl}_2$ . They were then propped up in their middle portions, with the ends dipping into separate pools of Ringer's solution in depression slides. The arrangement was as nearly symmetrical as

possible. At the end of each experiment, the nerves were lumped and assayed in the usual manner, and in addition the amount of radioactive substance that had appeared in each pool was determined.

One experiment comprising 6 guinea-pig nerves (4 hr.) gave a strikingly positive result and may be reported in detail (table 6). Besides the counts obtained from the lumped nerve samples, the table lists the contents of the twelve pools—one at the proximal ( $A_p$ ,  $B_p$ ,  $C_p$  . . .), and one at the distal ( $A_d$ ,  $B_d$ ,  $C_d$  . . .) end of each of the six nerves. It can be seen that the concentrations within the nerves were again much higher on the distal than on the proximal side. As for substance that had left the nerves, traces of it were present in all proximal pools. Traces of the same order were recovered from the distal pools of three of the nerves (A, B, C). In the remaining three nerves (D, E, F), however, the amounts of substance discharged from the distal ends were about 10 times as large as those discharged from their proximal ends. Thus, whenever

TABLE 6

*Distribution of  $Cu^{64}Cl_2$  in six excised guinea-pig nerves and in pools of Ringer's solution bathing the nerve ends*

CONCENTRATION WITHIN NERVE (QUANTA/MIN.)								CONCENTRATION IN POOLS (QUANTA/MIN.)						
Level	0	1	2	3	4	5	6	Nerve	A	B	C	D	E	F
D	12077	2950	659	397	153	163	76	D	10	5	12	33	66	89
P	12077	3794	245	27	10	5	10	P	7	5	7	3	7	9
D-P		-844	414	370	143	158	66	D-P	3	0	5	30	59	80
Asymmetry		D<P	D>P	D>P	D>P	D>P	D>P	Asymmetry	D~P	D=P	D~P	D>P	D>P	D>P

any oriented convection of substance occurred at all, it was in the proximo-distal direction.

Two other experiments with 4 rat nerves each yielded very low counts without significant asymmetry, either within the nerves or between the pools.

In a second series of tests, the excised nerves, uninjected, were placed in moist chambers with one cut end dipping into a solution of radioactive  $CuCl_2$  and the other end in ordinary  $CuCl_2$  (both concentrations 10 mgm./cc.). In half of the specimens, the distal end was exposed to the active solution; in the other half, the active solution was at the proximal end. The nerve was freely suspended and formed the only communication between the two pools.

One experiment (8 guinea-pig nerves, 7 hr.) gave the following results. The four nerves with the distal end in an active pool issued a weak radiation (cca. 10 quanta/min./cm.), slightly grading off towards the proximal end. Traces of the substance had actually passed into the proximal pools (7, 7, 9 and 12 qu./min., respectively). In contrast, the four nerves with the proximal ends in the activated solution, contained twice as much substance, and samples of 1 cm. length taken near the middle gave counts five times as high as the corresponding values of the former group. Evidently, more substance is taken up

through a proximal than through a distal cross section, supporting the thesis of a polarized proximo-distal convection inside the nerve. As for the four distal pools, three showed mere traces of transferred substance (4, 8 and 9 qu./min.), but one pool, significantly, gave the relatively high count of 33 quanta per minute. Thus, appreciable transfer has been observed in the proximo-distal direction, but in no case in the reverse sense. Two further experiments executed in the same manner but terminated earlier (after 3 hr.) gave negative results, as the time had obviously been too short for any appreciable transfer. The interpretation of these experiments is complicated by the fact that some of the substance presumably enters the axis cylinders themselves through the cut ends (Perdrau, 1937).

*E. Assay of blood.* No attempt was made to determine the time when the injected isotopes first appear in the blood stream. However, blood samples taken between 4 and 24 hours after injecting the nerves with either sodium chloride or copper chloride gave off radiation, increasing from an average of 30 quanta per minute per cubic centimeter of blood after four hours to 120 qu./min./cc. after 20 hours in the rat. The concentrations in the guinea pig were lower, presumably because of the greater blood volume.

**DISCUSSION.** The reported experiments have established the fact that, when minute amounts of a diffusible substance are deposited between the fibers of a nerve which is otherwise left intact, and after sufficient time has been allowed for the spread of the substance in the nerve, more substance is generally found distal to the injection point than proximally. This fact has been verified in several hundreds of cases, using ink, Prussian Blue, or radioactive isotopes as test substances.

To explain this asymmetry of distribution, one must assume either that the structural conditions for diffusion are different up and down a nerve or that diffusion is symmetrical, but the diffusion field as such is being carried downward. Structural conditions within the tested stretches of nerve were sufficiently uniform to discount the former explanation: diffusion in nerves injected sufficiently late after death is symmetrical (table 1). This leaves as the sole explanation a descending shift of the whole fluid in which the diffusion takes place.

That this descending fluid convection occurs in the endoneurial spaces has been demonstrated microscopically by the endoneurial position of ink particles and Prussian Blue granules in the respective experiments. While traces of sodium or copper in the isotope tests may have permeated into the nerve fibers themselves, the amounts would be negligible in comparison with the masses lying in the endoneurium. The scene of the observed convection effect is, therefore, the space between, rather than inside, the nerve fibers.<sup>2</sup>

<sup>2</sup> As a note of historic interest, we add here a translation of Ranvier's comment on the anatomical basis of the spreading of substances in nerve (*Traité technique d'histologie*; Paris, Librairie F. Savy, 1875):

"In 1824 Bagros (*Mémoire sur la structure des nerfs*; *Répertoire d'Anatomie et de physiologie*. vol. 4: 63, 1827) injected nerves with mercury. . . . He concluded that in the interior of each nerve fascicle there is a preformed canal analogous to a vascular canal. Later, Cruveilhier, . . . suggested that in injecting into the center of a nerve, one injects



Origin, composition and destination of the "endoneurial fluid" (Weiss, 1943a) are still unknown. It may be in communication with the subdural cerebrospinal fluid and receive contributions from blood capillaries, nerve fibers and epineurial lymphatics.

The normal rate of "endoneurial flow" cannot be safely calculated from our measurements, because it is perceptibly altered by the very indicators used to measure it. For instance, it is slowed by ferrocyanide injection (figs. 1 and 2), presumably due to an astringent effect on the endoneurial connective tissue. The isotope markers, on the other hand, tended to become bound to the tissue in less soluble compounds, thereby losing mobility. Even one day after an injection, the isotope, introduced in very soluble compounds, was still found near the injection site in relatively high concentrations. A general estimate of the rate of flow can, nevertheless, be obtained from experiments of short duration. The total shift of the diffusion field distad is given by the distance between the original injection point and the center of distribution of the marker substance at the end of the experiment. This shift, calculated from the graphs, figures 5 and 6, amounts to 10-15 mm. in 4 hours, or an average of cca. 3 mm. per hour. The same value has been found during the first hour after ferrocyanide injection (fig. 1), while after the implantation of ferrocyanide crystals, the rate was slightly less (cca. 1 mm. per hr.). These values, obtained by very different methods of injection and assaying and with different chemicals, are sufficiently in agreement to permit us to set the rate of endoneurial flow in the nerves studied as of the order of from one to a few millimeters per hour (one to several inches per day).

The mechanism of propagation and the motive force of this endoneurial flow are obscure. In an earlier paper (Weiss, 1943a), the hypothesis had been considered that the pulse wave might serve as motor. However, the observation that the flow may continue after stoppage of circulation, as well as in completely excised nerves, disposes of that hypothesis. The latter cases, at the same time, prove that the mechanism must lie and operate right in the nerve itself. In excised nerves, the distal convection effect was much less regular, and if present, usually weaker. This suggests that some uncontrollable by-product of transection may interfere with the mechanism of propagation; e.g., occlusion of the nerve interior by closure of the epineurium over the wound; superficial drying of the wound; presence or absence of blood clots; tufting of cut nerve fibers.

Pending further research, the problem remains a matter for speculation. It seems that a simple polarized convection of fluid in such a system as the interstices of nerve at the observed rate can be explained either by electrical

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neither the neurilem, nor the nervous substance, nor the vessels, but a sheath belonging to each fiber bundle ("filet"). I (i.e., Ranvier) have demonstrated since (*Recherches sur l'anatomic et la physiologie des nerfs*, Arch. de physiol., 1872, p. 439) that the injected mass which penetrates into a nerve fascicle spreads between the different constituent tubes and may extend longitudinally between them to great lengths without reaching the enveloping perineurium, and consequently the injection of nerve filaments does not imply the existence of a circumscribed canal in their interior" (pp. 770-771).

polarity or by mechanical propulsion. There is no crucial evidence in our experiments either for or against the assumption that the transport may be of electrophoretic nature (Yuien and Sato, 1929). One would have to postulate a steady and rather high electrostatic potential along the axis of the nerve as motive force. However, information on this latter point is scanty. Mendelssohn (1885) has described a longitudinal potential gradient in resting nerve. But we are unaware of any confirmatory evidence that would have been published since. Burr, in a private communication, informs us that exploratory tests on two uninjured sciatic nerves of guinea pigs *in situ* showed potential differences between two levels 8 mm. apart of the order of a few millivolts, with the distal level positive to the more proximal one. The "demarcation potential" after transection could be shown to be composed of the injury potential and the basic longitudinal potential of the intact nerve. Suggestive as these observations are, they are evidently not yet sufficient to support an electric theory of endoneurial flow.

Mechanical propulsion in narrow spaces may result from ciliary activity, which is definitely absent in nerve, or from peristaltic contractions. A slight peristalsis in nerve fibers, consisting of a rhythmic local dilatation and contraction wave propagated centrifugally, could readily produce the observed flow. In fact, the model suggested previously (Weiss, 1943a) for the collateral propulsion of endoneurial fluid by the pulse wave, is pertinent to any sort of peristaltic wave. It illustrates how a succession of such waves in closely packed tubular spaces would maintain a steady flow of liquid in the interstices, provided the whole system is contained in a rather rigid sheath as is the case with nerve.

The notion of peristalsis in mature nerve fibers deserves serious consideration. Some recent discoveries on the growth and behavior of constricted nerve fibers may have an intimate bearing on the case, as follows. Extensive observations on the damming, ballooning, telescoping and coiling of nerve fibers proximal to a constriction, briefly mentioned in previous publications (Weiss, 1943b, 1944a, 1944b), but not yet reported in full, have led to the conclusion that axonal substance is constantly moving at a very slow rate in centrifugal direction, not only in the growing phase, but in the stationary condition of the functional mature fiber. It may be difficult to explain this phenomenon otherwise than by some sort of peristalsis. A semblance of peristalsis can be discerned in some of Speidel's motion pictures of living axon sprouts. However, it has never been seen or suspected in the mature nerve fiber. If it should prove to be a fact, it could account for the endoneurial flow, too.

How the endoneurial fluid is disposed of peripherally, remains to be determined. Its biological significance is likewise still a matter of conjecture. Since it bathes all nerve fibers, its composition and physico-chemical properties are evidently of prime importance to the normal maintenance and functioning of nerve. Its possible rôle in nerve regeneration has been mentioned on previous occasions (Weiss, 1943a, 1943b).

The direct demonstration of centrifugal endoneurial flow in this paper confirms the interpretation of edema in constricted nerves advanced on an earlier occasion

(Weiss, 1943a; Weiss and Davis, 1943). It can readily be seen that any narrowing of the endoneurial channels must lead to accumulation of fluid between the nerve fibers at the proximal side of the constriction with resulting distention of the nerve. A similar edema often arises at the blind end of an unconnected central nerve stump, where further fluid transport is blocked by the connective tissue cap forming over the wound. Since such terminal edemas commonly form the basis for the development of large bulbous neuromas (Weiss, 1943a), it would seem indicated in amputations to insure conditions that will least interfere with the continued drainage of the nerve fluid into the surrounding tissues.

Denny-Brown and Brenner (1944) have contended that "edema" in constricted nerves is due to ischemia and may be present on both sides of a constriction. The latter statement is meaningless in view of the fact that the authors use the term "edema" for both the collection of interstitial fluid between nerve fibers and for the pathological swelling of nerve fibers themselves, which is a wholly different process. To avoid further confusion, the reader may be referred to microphotographs of typical cross sections of nerves proximal and distal to a constriction, reproduced in figure 4 of Weiss and Davis (1943) and figure 5 of Weiss (1943a), showing the strict confinement of interstitial edema to the proximal side. The notion that constriction edema in nerve is of vascular origin, has been advocated by Denny-Brown and Brenner as a matter of opinion, without experimental verification. It can hardly be accepted in the face of the experimental facts cited by Weiss (1943a) as disproving it. The alternative interpretation of nerve edema as dammed up endoneurial fluid was correctly labelled by Denny-Brown and Brenner as a "hypothesis." The facts reported in the present paper, however, remove it from that category.

Our demonstration of descending convection in the endoneurium seems to be at variance with earlier claims (see the introductory pages) according to which substance injected into nerve trunks spreads toward the cord. However, the two sets of data are hardly comparable. While we have used hundreds of animals, most earlier authors based their conclusions on a few, and not always consistent, cases. Where we used minimal amounts of substance (cca. 0.1 cu. mm.), exercised meticulous care to avoid major nerve disruption, controlled or wholly eliminated pressure and directiveness in the act of injection, and assayed the spread of the test substances quantitatively, they injected several thousand times as much, thereby causing profound disturbances of the nerve structure, took no adequate precautions against pressure artifacts, and judged their results by much cruder criteria. In those earlier experiments, so much violence was done to the nerve that the delicate phenomenon of endoneurial flow could not possibly have manifested itself.

While our experiments contain no indications that fluid traffic between fascicles and inside fascicles would occur in opposite directions, it should be borne in mind that the choice of small and poorly fasciculated nerves for our experiments means that our conclusions are strictly applicable to the endoneurial, i.e., intrafascicular transport only.

## SUMMARY

1. The hypothesis of a steady proximo-distal movement of fluid in the endoneurial spaces of peripheral nerves, first suggested by observations on nerve edema (Weiss, 1943a), was subjected to an experimental test, comprising a total of 420 limb nerves of rats and guinea pigs.

2. To test fluid transport in nerve, marker substances were deposited between the nerve fibers with the least possible damage to the continuity and structure of the nerve. In contrast to earlier experiments of this kind, only minute amounts of substance were used (*ca.* 0.1 cu. mm.) and care was taken to eliminate all possible artifacts due to the pressure and orientation of the injection. Test substances were introduced either in solution or in solid form. After periods varying from less than one hour to a few days, the distances to which the substance had spread up and down the nerve were determined. With the use of radioactive tracers, the actual shape of the concentration gradients could be determined. These assays gave the following results.

3. Diffusion in nerves injected several hours after death proceeds symmetrically, that is, for equal distances up and down from the injection point.

4. In live nerves, no matter what method is used, an excess of substance is found distal to the site of injection, indicating a progressive transport of the injected substance in the distal direction, with endoneurial fluid serving as vehicle.

5. India or Chinese ink injections (46 expts.) showed the distal shift but were not fully conclusive because of technical difficulties.

6. Injection of potassium ferrocyanide which was later precipitated to Prussian Blue by ferric chloride (162 cases) proved, on the whole, the presence of a proximo-distal shift in intact nerves at a rate of from 1 to 3 mm. in the first hour, gradually slowing down, presumably because of an astringent action of the test substance. In transected nerves, the convection effect is more or less disturbed.

7. Injection of radioactive isotopes ( $\text{Na}^{24}\text{Cl}$ ,  $\text{Cu}^{64}\text{Cl}_2$ ) made it possible to follow the distribution of the injected material quantitatively with the aid of a Geiger-Müller counter. The results obtained with 193 nerves thus studied have proved conclusively the gradual shift of the diffusion gradient in the distal direction, indicating a proximo-distal flow of the endoneurial fluid at the rate of a few millimeters per hour. This flow may continue after the stoppage of circulation and even in completely excised nerves, although nerve transection often introduces uncontrollable disturbances.

8. The origin and fate of the endoneurial fluid and the mode and motive force of its displacement down the nerve are unknown. An electrical and a mechanical concept (peristalsis of nerve fibers), both wholly tentative, are briefly discussed in the text.

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# THE EFFICIENCY OF THE GLARE REDUCTION BY THE EYELIDS

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Protection against glare is an important function of the eyelids. When looking with closed eyes against a bright light source, for instance sun light, there is still a sensation of intense brightness, so that subjectively the reduction of glare is far from complete. The effectiveness of the eyelids in glare reduction has theoretical and practical significance. To our knowledge, no attempt has yet been made to determine this function. We were interested in measuring the effects on clearly defined visual functions rather than on the subjective glare sensation. It is known (1) that objective glare effect and subjective glare sensation are not necessarily parallel. The best approach to determine the proportion of light penetration through the eyelids appeared to be the use of light and of dark adaptation. Dark adaptation was useful in a comparative study of the glare effect of two different illuminants (2), the duration of dark adaptation being proportional to the level of brightness of exposure. An even better method is the speed of light adaptation, since the decrease of sensitivity of the retina during light exposure is the primary function and the recovery of sensitivity (dark adaptation) is the secondary function.

**METHOD.** Light adaptation has been considerably less studied than dark adaptation. While there are several studies concerning the influence of the brightness level on light adaptation, we know of only one paper (3) dealing with the effect of varying duration of exposure between one and forty minutes, i.e., the speed of light adaptation. These time intervals are too long to determine the course of threshold changes during light adaptation, especially in the early phases where the speed changes rapidly. We have, therefore, measured the speed of light adaptation with time intervals of seconds rather than minutes; this material will be published separately. Elsberg and Spotnitz (4) varied duration of exposure in shorter intervals, but they used as a criterion of dark adaptation the threshold of visual discrimination at a level of illumination which, although dim, was far above the range used for dark adaptation experiments. They found that the duration of exposure is a more important factor than the level of brightness. Although dark adaptation probably plays a major rôle in their arrangement, other visual functions are included also, so that their results are not conclusive.

Blanchard (5) has worked out an accurate method to measure the instantaneous threshold after light exposure. The results were discussed and appreciated by Hecht (6). Immediately after exposure to light, the subject views the test patch of a dark adaptometer. After several trials, the brightness of the test patch can be adjusted so that it can be just recognized immediately after

exposure. It is clear that 5 to 6 trials, at least, are necessary to determine the instantaneous threshold value. Since we compared three different illuminants with exposure periods from 2 to 300 seconds, resulting in a number up to 40 exposures in one single series, Blanchard's original method was not applicable. Instead, we used an approximate method, measuring the threshold about 5 seconds after the light exposure in successive experiments with increasing light exposure which we define as relative light adaptation threshold. Thus, we obtained a curve of threshold values. These values are somewhat below the instantaneous threshold, but definitely and closely related to it. Certainly, the values are comparable so that the calculation of the percentage of light penetration through the eyelids is not affected.

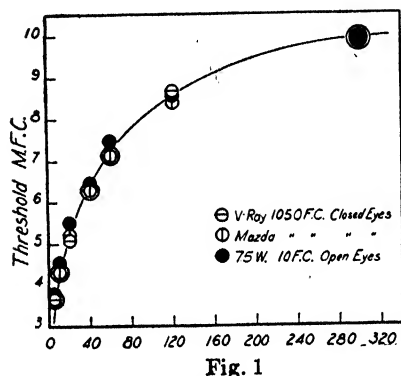


Fig. 1

Fig. 1. Light sensitivity, thresholds in M.F.C., after exposure of increasing duration (abscissa, seconds) to a dim (10 F.C., eyes open) and a bright light (1050 F.C., two illuminants, eyes closed).

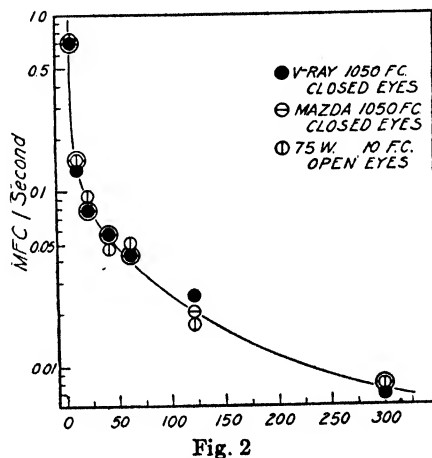


Fig. 2

Fig. 2. Speed of light adaptation, M.F.C. per second, after exposure of increasing duration (abscissa, seconds) to a dim (10 F.C., eyes open) and a bright light (1050 F.C., two illuminants, eyes closed).

The simplest procedure for the calculation of the percentage of light penetration would be to expose open and closed eyes to the same light source, and to vary the exposure time in such a way that either the relative light adaptation threshold (first reading after 4 to 5 seconds) or the adaptation time (last reading) for both arrangements (open and closed eyes) are equal. This procedure, however, is not accurate, because the light adaptation speed changes considerably during the light adaptation. Since the light adaptation speed also depends on the brightness level, different results were obtained at different levels of brightness. Therefore, we decreased the brightness of the exposure in the arrangement with open eyes, until the speed of light adaptation was approximately the same with closed and open eyes. We matched the brightness for subject S. S.; figure 1 shows that the course of light adaptation is about the same with open eyes and with closed eyes with two different illuminants. The

other two subjects were investigated at the same standard conditions as subject S. S. Since we were interested only in an approximate estimation of the percentage of light penetration within the range of a few percent—any greater accuracy would be indeed quite meaningless due to intra- and interindividual variations—this procedure was sufficiently accurate. For exposure to open eyes we used frosted glass (Mazda) bulbs (lamp A) and Verd-A-Ray bulbs (lamp B). In illuminant B, the radiation at both ends of the visual spectrum is reduced without appreciable reduction in the middle part of the spectrum (between 5400 and 5600 AU<sup>1</sup>).

Three well trained subjects were used (S. B., S. S., E. S.). For light exposure the subject viewed an illuminated frosted glass plate (8 by 8 inches) from a distance of 6 (lamp A) or 3 inches (lamp B). The visual field, therefore, was very large. The time of successive exposures was 2, 5, 10, 20, 40, 60, 80, and 100 seconds for subject S. B.; 5, 20, 60, 120, and 300 seconds for subject E. S.; 5, 10, 20, 40, 60, 120, and 300 for subject S. S. The brightness was 1050 F.C. in the arrangement with closed eyes and 10 F.C. with open eyes. Immediately after exposure the subject turned over to the dark adaptometer. The experimenter increased the brightness of the test patch until recognition. Since preliminary experiments had given the approximate range of expected initial threshold values, it was possible, after some training, to have this reading taken 4 to 5 seconds after the end of the exposure. These readings could be repeated with an error not exceeding  $\pm 5$  per cent. The time when the reading was taken was noted by means of a stopwatch, but little difference was found when the reading time was taken within one second. After this first reading the dark adaptation was measured until the value of 0.05 M.F.C. was reached. This value includes full cone adaptation and the first (rapid) segment of rod adaptation. For our problem, cone adaptation is much more important than rod adaptation. Since light adaptation is a pure cone function with the levels of brightness used in our arrangement, only cone dark adaptation can be related to the correspondent values of light adaptation.

We used Newton's instrument, where the brightness of the test patch (of  $\frac{1}{2}$  degree, 5 degrees above the small red fixation point), is varied by means of variation of the angle of the beam illuminating the test patch. Thus the color of the test patch does not change at different brightness levels. On each experimental day, all three illuminants were investigated (lamp A and lamp B, closed eyes 1050 F.C.; 10 F.C. lamp A, open eyes), but their sequence was changed in the consecutive experimental days. Each arrangement was used twice in the same order in the total of 18 experiments in each subject. There was no evidence, however, that the sequence was of importance for the results obtained. For calculation, only the data with lamp A, closed eyes at 1050 F.C. and open eyes at 10 F.C. were used, since both arrangements concern usual frosted lamp bulbs. From the increasing threshold values with increasing duration of light exposure, the average speed of light adaptation for the consecutive periods was

<sup>1</sup> More detailed data on the spectral distribution of this illuminant are given in an earlier paper (7).



calculated in terms of M.F.C. per second. From the values, the percentage of light penetration through the eyelids was calculated for each subsequent period, so that the influence of the change of the light adaptation speed with exposure

TABLE 1

*Light adaptation (threshold M.F.C. and speed: M.F.C. per second) and coefficient of light penetration through the eyelids*

SUBJECT	FUNCTION	TIME OF LIGHT EXPOSURE										ILLUMINATION	
		2	5	10	20	40	60	80	100	120	300		
		sec.	sec.	sec.	sec.	sec.	sec.	sec.	sec.	sec.	sec.		
S. B.	Threshold M.F.C.	3.43	3.98	4.71	5.85	7.52	8.65	9.58	10.0			Lamp B, 1050 F.C., closed eyes	
	Light adaptation speed	1.71	0.181	0.146	0.114	0.083	0.066	0.046	0.021				
	Threshold M.F.C.	3.18	3.97	4.68	5.60	7.39	8.62	9.80	10.0			Lamp A, 1050 F.C., closed eyes	
	Light adaptation speed	1.59	0.261	0.142	0.098	0.089	0.061	0.059	0.01				
	Threshold M.F.C.	4.60	5.75	7.15	8.65	10.0						10 F.C. open eyes	
	Light adaptation speed	2.30	0.383	0.28	0.15	0.067							
	Per cent light penetration	0.691	0.622	0.481	0.621	1.263							
S. S.	Threshold Light adaptation speed		3.67 0.734	4.30 0.134	5.07 0.077	6.25 0.059	7.13 0.044			8.65 0.0253	9.92 0.007	Lamp B, 1050 F.C., closed eyes	
	Threshold Light adaptation speed		3.60 0.72	4.37 0.154	5.17 0.08	6.27 0.055	7.13 0.043			8.38 0.021	9.88 0.008	Lamp A, 1050 F.C., closed eyes	
	Threshold Light adaptation speed		3.80 0.76	4.55 0.15	5.50 0.095	6.45 0.0475	7.48 0.051			8.52 0.0174	9.92 0.008	10 F.C., open eyes	
	Per cent light penetration		0.92	0.978	0.805	1.10	0.805			1.14	0.95		
E. S.	Threshold Light adaptation speed		1.96 0.392		3.24 0.086		4.92 0.039			6.92 0.033	9.92 0.016	Lamp B, 1050 F. C., closed eyes	
	Threshold Light adaptation speed		1.72 0.344		2.96 0.083		4.68 0.043			7.16 0.041	9.68 0.014	Lamp A, 1050 F.C., closed eyes	
	Threshold Light adaptation speed		2.48 0.496		3.56 0.072		5.68 0.053			6.80 0.0189	8.16 0.008	10 F.C., open eyes	
	Per cent light penetration		0.661		1.011		0.773			2.13	1.745		

time is largely eliminated. In case the light adaptation speed in a given period is identical with closed eyes (1050 F.C.) and with open eyes (10 F.C.), the penetration coefficient is obviously  $10/1050 = 0.0095 = 0.95$  per cent. If the speed is slower with closed eyes ( $L_c$ ) than with open eyes ( $L_o$ ) a smaller per-

centage of light than 0.95 per cent has penetrated the eyelids; if the speed is greater with closed eyes, the percentage of light penetration is greater than 0.95 per cent. Therefore, the penetration coefficient is  $0.95 \times \frac{LC}{LO}$ . A similar calculation was made for the cone dark adaptation periods, using the total duration necessary to recognize the final threshold of 0.05 M.F.C.

RESULTS. Table 1 shows the immediate thresholds, i.e., 4 to 5 seconds after exposure of increasing duration, each value representing the average of six experiments. The increasing thresholds show the course of light adaptation

TABLE 2

*Cone dark adaption time (seconds) and coefficient of light penetration through the eyelids*

SUBJECT	FUNCTION	TIME OF LIGHT EXPOSURE (seconds)										ILLUMINATION
		2	5	10	20	40	60	80	100	120	300	
S. B.	Dark adapt. (seconds)	8.67	11.7	14.5	19.0	22.7	32.5	42.2	43.5			Lamp B, 1050 F.C., closed eyes
	Dark adapt. (seconds)	9.00	11.5	14.7	16.2	24.2	32.5	38.4	50.5			Lamp A, 1050 F.C., closed eyes
	Dark adapt. (seconds)	13.2	17.7	26.0	32.8	44.0						10 F.C., open eyes
	Per cent penetr.	0.65	0.65	0.54	0.495	0.52						
S. S.	Dark adapt. (seconds)		6.8	9.0	10.0	13.3	14.0			19.3	17.4	Lamp B, 1050 F.C., closed eyes
	Dark adapt. (seconds)		8.0	9.0	9.4	9.6	12.8			15.7	16.7	Lamp A, 1050 F.C., closed eyes
	Dark adapt. (seconds)		8.8	11.2	12.2	13.8	18.7			25.7	32.0	10 F.C., open eyes
	Per cent penetr.		0.86	0.77	0.73	0.66	0.68			0.58	0.50	
E. S.	Dark adapt. (seconds)		7.2		19.2		31.0			36.2	45.4	Lamp B, 1050 F.C., closed eyes
	Dark adapt. (seconds)		8.4		13.2		26.4			31.8	42.6	Lamp A, 1050 F.C., closed eyes
	Dark adapt. (seconds)		12.0		23.2		39.4			59.4	99.4	10 F.C., open eyes
	Per cent penetr.		0.67		0.54		0.64			0.53	0.42	

with exposure time (s. fig. 1), rapidly increasing during the first seconds and then gradually slowing down, approximating a steady state. From these values, the speed of light adaptation was calculated in terms of M.F.C. per second, shown in the second horizontal columns of table 1, and in figure 2, using a logarithmic scale. The last column shows the percentage of penetration, as calculated from lamp A, 1050 F.C., closed eyes, and 10 F.C., open eyes. Due to the much faster light adaptation speed of subject S. B., shorter exposure times (from 2 to 100 seconds) were given. The penetration coefficient of subject S. B. is between 0.5 to 0.7 per cent up to 20 seconds' exposure and increases to 1.263 per cent at 40 seconds. Since 10 M.F.C. represents the highest meas-

urable value of our adaptometer and since this value was reached with open eyes at 40 seconds, no values beyond 40 seconds are available for comparison. For subject S. S., the percentage of penetration is about the same (about 1 per cent) through the whole range from 5 to 300 seconds exposure time. For subject E. S., the variation is somewhat greater with a tendency to increase at the longer exposure time. In spite of individual differences of light adaptation speed, the percentage of penetration shows surprisingly small individual variations and is in the range of approximately 1 per cent.

Table 2 shows the results of cone dark adaptation time until recognition of 0.05 M.F.C. The percentage of penetration, calculated from these data, is lower than that obtained from light adaptation, varying between 0.5 and 0.8 per cent, with a tendency to decrease with increasing exposure time. Again, the similarity of results is surprising in spite of considerable individual differences of individual dark adaptation, which is much faster in subject S. S.

COMMENT. The efficiency of the glare protecting function of the eyelids is rather high; approximately 1 per cent (calculated from light adaption) or approximately 0.6 per cent (calculated from cone dark adaptation) of the total light flux penetrates the eyelids. Considerable individual differences of light and of dark adaptation influence the magnitude of this penetration coefficient but little. This result is quite in contrast to the subjective glare effect, which is still considerable when looking with closed eyes against 1050 F.C., and certainly greater compared to the subjective brightness sensation with open eyes at 10 F.C. Obviously, the physiologically effective radiation as measured by means of dark and light adaptation differs from the subjectively effective radiation. There is reason to believe that the spectral distribution is changed by the passage through the eyelids, probably by the blood circulating in the eyelids. The after images in the experiments with open and with closed eyes differ in that the color is green after light exposure with closed eyes. Furthermore, no difference in light and dark adaptation with closed eyes was found between illuminant A and illuminant B in this series, although illuminant B shows faster dark adaptation and slower light adaptation (2) with open eyes, as evidence of a less pronounced glare effect. Since the spectral distribution of illuminant B differs from illuminant A (s. method), this difference must be nullified by the passage through the eyelids. Another factor of importance is the scatter effect by the eyelids; when moving the eyeballs with closed eyes in our arrangement, the visual field appears to be equally bright in every direction.

The different magnitudes of the penetration coefficients for light and for dark adaptation are obviously due to the fact that light and dark adaptation are not merely processes different in direction, but also different in nature. Subject S. B. has the fastest light adaptation speed; subject S. S. has the fastest dark adaptation speed. This shows that it is impossible to match in one arrangement the speed of light and of dark adaptation simultaneously by variation of the brightness for open or closed eyes even for the same subject. This explains also the different trends, especially of dark adaptation at different exposure times. An important suggestion from these experiments, although not directly

concerned with our results, is that light adaptation should be considered in all experiments with dark adaptation. Usually, different subjects are compared with the same standard light exposure, but, as shown in table 1, large individual differences of light adaptation do exist, so that dark adaptation might start after the same light exposure, from a very different initial threshold in various subjects.

#### SUMMARY

Light and dark adaptation were used to measure the coefficient of light penetration through the eyelids, after exposure times varying from 2 to 300 seconds. Only approximately 1 per cent of physiologically effective light calculated from light adaptation, and 0.6 per cent calculated from dark adaptation penetrates the eyelids. The values of three well trained subjects were similar, in spite of considerable individual differences in light and dark adaptation speed.

The high efficiency of the glare protecting function is probably to a large extent due to a change of spectral distribution during the passage through the eyelids.

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# EFFECT OF ADRENOCORTICOTROPIC HORMONE ON THE SURVIVAL OF NORMAL RATS DURING ANOXIA<sup>1</sup>

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From the works of Evans (1), Armstrong (2) and Thorn (3), it is apparent that the adrenal cortex plays an important rôle in promoting the survival of rats under anoxic conditions. However, no adrenal therapy has hitherto been shown to benefit *normal* rats exposed to experimental "high altitudes." The experiments herein reported show that when the adrenals of rats were hypertrophied from the injection of pituitary adrenocorticotrophic hormone, such animals lived much longer than those without this pre-treatment.

**EXPERIMENTAL.** The adrenocorticotrophic hormone (ACTH) was prepared from sheep pituitaries by the method recently described (4). Forty to 43 day old normal male (Long-Evans strain) rats were given adrenocorticotrophic hormone intraperitoneally twice or thrice daily for 7 days. On the day of the anoxia test, 6 or 7 injections were made at approximately two hour intervals. The rats were then placed in individual low pressure chambers and decompressed at a rate equivalent to an ascent of 1,500 ft. per minute to a plateau of 27,000 ft. (258 mm. Hg). A thermostatically controlled cabinet kept the environmental temperature 25° to 26.5°C. Unless otherwise specified the preceding diet was fed ad libitum. The rats remained in the low pressure chamber without food or water until death. An autopsy was performed on each rat.

The low pressure equipment devised in this laboratory accommodates 40 rats in individual chambers thus providing for the immediate removal of any rat at the exitus lethalis for purposes of autopsy, etc., without disturbing others. Chambers were made of quart fruit jars with floors of wire screen and two-piece metal lids through which were soldered  $\frac{1}{4}$  inch copper tubing inlet and outlet tubes. A heavy vacuum seal grease was used: there was a complete absence of leaks at the equivalent of 40,000 ft. elevation. The inlet and outlet tubes of the chambers were connected by short rubber vacuum tubing to  $\frac{1}{8}$  inch pipe brass stopcocks threaded into half inch water pipe. A needle valve at the inlet and outlet of the vacuum line was used to balance the incoming and outgoing air for 20 chambers. A mercury manometer registered the vacuum. The vacuum source was very constant and gave fluctuations of less than  $\pm 100$  ft. in 27,000 ft. equivalent elevation. The air inflow to each chamber renewed the air in excess of eight times per minute.

**RESULTS.** As will be seen in table 1, the animals having pre-treatment with the adrenocorticotrophic hormone lived longer than untreated controls. Column 5 of the table gives the number of rats alive in excess of those alive in the un-

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treated control group; it may be noted that it required a total dose of 4.5 mgm. of the hormone for total protection under the conditions of the experiment.

TABLE 1

*Effect of pure pituitary adrenocorticotrophic hormone on the survival of normal rats during anoxia\**

TOTAL DOSE HORMONE	NO. OF RATS	BODY WEIGHT	AVERAGE SURVIVAL	NO. OF RATS ALIVE BEYOND CONTROLS	WT. OF 2 ADRENALS	WT. OF THYMUS
<i>mgm.</i>		<i>grams</i>	<i>hrs.</i>		<i>mgm.</i>	<i>mgm.</i>
63.0	9†	59	12.5	7	40	30
0.0	9†	69	3.1	0	21	185
21.0	7	160	10.1	6	72	138
0.0	6	165	2.5	0	38	392
5.0	10	172	6.7	10	46	399
0.0	10	174	3.0	0	38	311
4.5	10	159	19.9	10	56	192
1.8	10	162	3.4	4	46	279
0.5	8	166	9.8	2	42	360
0.0	7	164	1.5	0	30	430

\* Elevation, 27,000 ft. at 25–26°C.

† 28 day old male rats. All other groups are 40–46 days of age.

TABLE 2

*Effect of alkaline extract of beef anterior hypophysis, acid-acetone powder from sheep glands and pitressin on the survival of normal rats during anoxia\**

	TOTAL DOSE	NO. OF RATS	BODY WT.	AVERAGE SURVIVAL	NO. OF RATS ALIVE BEYOND CONTROLS	WT. OF 2 ADRENALS	WEIGHT OF THYMUS
	<i>mgm.</i>		<i>grams</i>	<i>hrs.</i>		<i>mgm.</i>	<i>mgm.</i>
Crude alk. extract..	71.5†	10	179	3.6	0	53	393
	32.5	10	182	3.9	1	38	361
	0.0	9	181	5.8	0	38	400
Acid-acetone powder	130†	10	145	18.5	3	58	127
	70	10	155	2.2	0	43	244
	0	10	178	4.7	0	39	488
Pitressin.....	5 units	5	153	3.0	0	37	323
	0	5	152	3.3	0	38	405

\* Elevation, 27,000 ft. at 25–26°C.

† Injections were carried out at 2 hour intervals beginning 16 hours before anoxia.

In some preliminary experiments in which we employed partially purified adrenocorticotrophic fractions, it was observed that the survival of injected animals was actually shortened although the adrenal was hypertrophied, but the

experiments herein reported with pure ACTH lead us to interpret the earlier results as due to some contaminant or contaminants of the impure preparations.

Experiments using the pressor principle of the posterior pituitary (Pitressin, Parke, Davis Co.) did not shorten the survival. These results and those from the employment of crude alkaline extract of beef anterior lobes and the acid-acetone powder of sheep pituitaries (starting material for the isolation of adrenocorticotrophic hormone) (4) are summarized in table 2. The acid-acetone powder, 10 mgm. daily for 7 days, produced no benefit and actually caused a shortening in survival. However, if the injections were administered at 2 hr. intervals beginning 16 hrs. preceding anoxia, the rats lived much longer than their con-

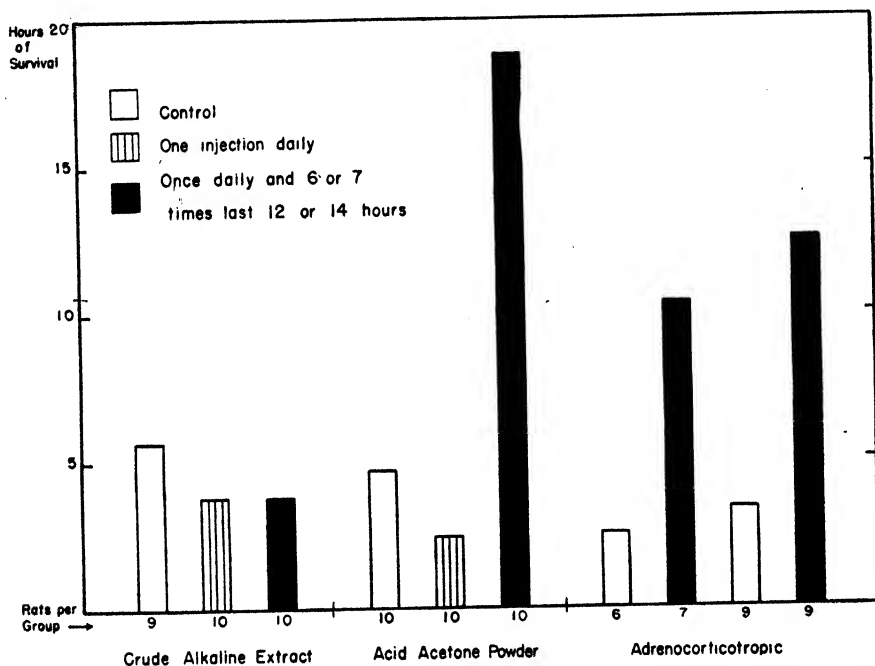


Fig. 1. The anoxia survival (in hours) of normal male rats receiving pituitary adrenocorticotrophic hormone (ACTH), acid acetone powder and alkaline extract of beef pituitary. Elevation, 27,000 ft. at 25-26°C.

trols. The crude alkaline extract evidently contains such injurious contaminants that in spite of hypertrophied adrenals, protection to anoxia was not afforded.

#### SUMMARY

Figure 1 summarizes the data on the anoxia survival of rats receiving pure adrenocorticotrophic hormone, pitressin, sheep pituitary acid-acetone powder and alkaline extract of beef pituitary. It is clearly shown that the adrenocorticotrophic hormone exercises a beneficial effect on the resistance of normal male rats to low atmospheric pressure.

We are deeply grateful for the encouragement given to us by Prof. Herbert M. Evans during this investigation.

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# THE USE OF CHOLINESTERASE IN SHOCK

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This paper presents the therapeutic effects of cholinesterase and plasma on hemorrhagic and traumatic shock. It was observed previously, but not reported, that during the manipulation of intestines of experimental hyperthyroid dogs to produce shock, an injection of eserine caused a precipitous fall in blood pressure. This phenomenon indicated that in the genesis of shock, the cholinesterase mechanism might play a rôle worthy of more complete study.

**EXPERIMENTAL METHODS.** Dogs anesthetized, except where noted, with 300 mgm. of sodium barbital per kgm. were used throughout these studies. Hemorrhagic shock was produced by repetitive bleedings from the femoral artery until shock was established. Traumatic shock was produced by manipulating a loop of intestine until the blood pressure dropped and remained at shock levels. In both types of shock, therapy (if any) was instituted only after the blood pressure remained at shock levels of 60 mm. Hg for at least 30 minutes.

*Hemorrhagic shock (I).* Normal anesthetized dogs were used. Continuous kymographic tracings of blood pressure were recorded from a carotid artery. Following a control period of 15 to 30 minutes, a volume of blood equivalent to 2 per cent of the dog's body weight was bled very rapidly from a femoral artery. After such a bleeding the blood pressure drops precipitously but in a few minutes partially recovers. After an interval of at least 15 minutes, additional blood to the extent of 1 per cent of the dog's body weight was withdrawn, and this was repeated until the mean blood pressure remained at 60 mm. Hg or lower for at least 30 minutes. Usually the dogs were bled 4 per cent of their body weights to attain this condition of shock. Hematocrit and serum cholinesterase values were determined during the control period and at intervals after the bleeding. The hematocrits were read in Van Allen tubes. Cholinesterase was determined according to Ammon's (1) method, which depends on the hydrolysis of acetylcholine to carbon dioxide and acetic acid by the cholinesterase. The actual determination was as follows: 0.25 cc. of serum was put in the central part of the Warburg vessel and 2.5 cc. of bicarbonate-Ringer buffer was added. The buffer was prepared by adding 21 cc. of 0.154 M  $\text{NaHCO}_3$  to 100 cc. of mammalian Ringer solution and the solution equilibrated for 5 minutes with a mixture of 95 per cent  $\text{N}_2$  and 5 per cent  $\text{CO}_2$  giving a pH of 7.4. Acetylcholine chloride, 5 mgm. dissolved in 0.25 cc. bicarbonate-Ringer solution, was placed in the side arm. The vessel was flushed with 95 per cent  $\text{N}_2$ -5 per cent  $\text{CO}_2$  at  $38^\circ\text{C}$ . for 10 minutes before closing the stopcocks and then the acetylcholine in the side arm was added. Readings were taken every 3 minutes for 18 minutes. One unit of cholinesterase is defined as that amount of enzyme which under these conditions gives 1 mm.<sup>3</sup> of  $\text{CO}_2$  per minute during the ensuing 3 to 18 minutes.

Hemorrhagic shock was produced in 18 dogs which make up 3 groups, according to the treatment given subsequently:

1. In 6 dogs no attempts at therapy were made.
2. In 6 dogs 3000 to 5000 units of cholinesterase, prepared according to the method of Stedman, Stedman, and Eason (2), were injected intravenously, after the blood pressure had remained at shock levels for at least 30 minutes.
3. In 6 dogs dried beef plasma, brought to the original volume by addition of distilled water, was injected intravenously after the blood pressure had been at shock levels for at least 30 minutes. The amount of beef plasma injected was equal to or greater than the volume of plasma but not of whole blood which the dog had lost by hemorrhage.

The results of these experiments with dogs show that cholinesterase does not alleviate the symptoms of shock produced by hemorrhage, while dried beef plasma is effective in raising and maintaining the blood pressure of these animals. Detailed analysis of these experiments is shown in table 1.

*Shock produced by gut manipulation (II).* Experimental hyperthyroid dogs were used in these studies, because Schachter and Huntington (3) observed that such dogs go into shock more readily than do normal dogs. The dogs were divided into 4 groups:

1. Serum cholinesterase was determined on 18 normal unanesthetized dogs, which were then rendered hyperthyroid by feeding 0.8 gram of desiccated thyroid per kgm. per day for 14 days. The serum cholinesterase on the 7th and 14th days of thyroid feeding was not appreciably different from the initial value. The results are shown in table 2.

2. Twenty-four experimental hyperthyroid dogs were anesthetized and the blood pressure was recorded from a carotid artery. Following a control period of about 15 minutes, the abdominal wall was opened and a loop of intestine taken out and manipulated between the palms of the hands for not more than 15 minutes. Such manipulation caused most animals to go into shock. The intestine was then replaced and the abdominal wall closed. If the blood pressure did not stay at the shock level, the manipulation was repeated. At no time did the total manipulation exceed 30 minutes. Serum cholinesterase and hematocrit values were determined before gut manipulation and during shock. All of these dogs died in shock (table 1).

3. Six hyperthyroid dogs were shocked as under 2. When the dogs had been in shock for about 30 minutes, intravenous injection of beef plasma was given. The amount of plasma administered was equal to the calculated plasma volume of the animal. In spite of the plasma given, all these dogs died in shock (table 1).

4. Eighteen dogs, thrown into shock as under 2, were treated with 3000 to 5000 units of cholinesterase instead of plasma. The cholinesterase was administered intravenously at the rate of 10 to 15 units in 0.1 to 0.15 cc. of fluid per minute. The administration of cholinesterase caused a gradual rise in blood pressure and an increase in plasma volume as indicated by the reduced hematocrit values. In fact the symptoms of shock were alleviated in 16 out of 18 dogs, as shown in table 1. Several hours after the cessation of cholinesterase adminis-

trations the blood pressures were still at normal levels, and presumably the dogs would have continued to live if the experiments had not been terminated after about 6 hours.

In order to test whether it was the cholinesterase or some other substance in the preparation which was responsible for the alleviation of shock symptoms, the following experiment was done:

Six dogs were fed thyroid, as previously described, and then shock was induced by intestinal manipulation. Four of these six dogs were treated with cholines-

TABLE 1

*Average results of experiments on experimental shock due to hemorrhage and trauma to the intestine, and the effect of therapy on the course of experiments*

TYPE OF SHOCK	NO. OF DOGS	TYPE OF THERAPY	AVERAGE HEMATOCRIT %			AVERAGE UNITS OF CHOLINESTERASE* PER CC. OF SERUM			DIED		SURVIVED	
			Be-fore shock	Dur-ing shock	At end of experi-ment	Before shock	During shock	At end of experi-ment	No.	Min.	No.	Remarks
Hemorrhage	6	None				31.7 ±3.15		30.5 ±3.13	6	187		
Hemorrhage	6	Infusion of cholinesterase	49	49	40	35.0 ±1.06	35.8 ±2.36	33.3 ±1.23	6	150		
Hemorrhage	6	Plasma	51	46	43	37.4 ±2.74	38.2 ±2.94	36.1 ±3.54			6	Terminated experiments on the average at the end of 218 minutes with blood pressure above shock levels
Gut manipulation	24†	None	50	54	59	34.7 ±2.21		37.4 ±2.18	24	150		
Gut manipulation	6†	Plasma	54	63	49	41.3 ±1.57	45.9 ±1.73	40.9 ±2.63	6	176		
Gut manipulation	18†	Infusion of cholinesterase	52	60	55	38.9 ±0.98	44.5 ±1.09	38.6 ±1.54	2	207	16	Recovered from shock and had normal blood pressures when the experiments were terminated after an average of 6 hours

\* The value given is the average  $\pm$  the standard error of the average.

† Dogs were fed desiccated thyroid 0.8 gram/kgm./day for 14 days prior to experiment.

terase originally equivalent to 5000 units but inactivated by heating in an oven at 100°C. for 3 hours. None of these dogs were benefited by the injection of the inactivated cholinesterase and all died in shock.

Another 2 dogs, after having been put into shock by gut manipulation, were treated with cholinesterase inactivated by physostigmine salicylate. These dogs died.

DISCUSSION. Blalock (4) and Parsons and Phemister (5) maintain that shock is due to a loss of plasma-like fluid at site of injury. From their work it would

be expected that replacing the lost fluid with either plasma or whole blood would be adequate therapy. However, this is generally not the case.

Duncan and Blalock (6), Best and Solandt (7) and Taylor and Moorhouse (8) found that injection of fluid into animals shocked by trauma or crush to the limbs would not save these animals. Clinically, Grant and Reeve (9) found that treating air-raid casualties with plasma did not save many patients from shock. The results of the studies reported in this paper confirm the above findings for,

1. Plasma alleviates the shock condition due to uncomplicated hemorrhage,
2. Plasma does *not* materially alter the shock due to trauma, and
3. Cholinesterase alleviates the shock syndrome due to trauma.

TABLE 2

*Units of cholinesterase per cubic centimeter of serum of unanesthetized dogs before and after feeding of 0.8 gram of desiccated thyroid per kilogram per day*

DOG NO.	BEFORE THYROID FEEDING	ONE WEEK AFTER THYROID FEEDING	TWO WEEKS AFTER THYROID FEEDING
1	29.8	34.2	32.7
2	34.4	30.3	34.5
3	29.8	26.4	26.4
4	41.6	42.9	45.7
5	51.2	53.1	52.1
6	36.2	45.8	43.3
7	41.7	41.5	48.1
8	38.3	39.5	37.2
9	40.8		43.3
10	33.0	35.7	37.1
11	25.9	34.7	33.1
12	35.9	42.1	43.9
13	28.8		31.3
14	43.8		44.3
15	39.6		35.8
16	41.3		47.3
17	32.9		25.2
18	44.9		44.2
Average.....	37.2	38.4	39.2

The *modus operandi* of cholinesterase is not clear. The serum cholinesterase is not diminished either during thyroid feeding or during shock, as is demonstrated in tables 1 and 2. For any one dog, the slight variation of serum cholinesterase after one and two weeks of thyroid feeding is of no significance, since the between-dog values vary so much. Therefore it would not seem that shock is due to a diminished serum cholinesterase. This does not agree with the findings of Frommel et al. (10), who found a decrease in serum cholinesterase activity following trauma to the legs of guinea pigs. On the other hand, there is no increase in the cholinesterase content in the serum shortly after the intravenous administration of cholinesterase. Of course the amount of cholinesterase administered is small compared with the amount normally present in

the dog's plasma; nevertheless, after injection of cholinesterase, an increase rather than the slight diminution shown in table 1 would be expected.

Since cholinesterase causes a reduction in the hematocrit values, its effectiveness in traumatic shock may be due to a shift in fluid from the tissues into the blood stream, with a resultant increase in plasma volume. Or the beneficial therapy of cholinesterase may be due to destruction of any increased acetylcholine in the blood stream consequent to the trauma; the acetylcholine would cause dilatation to such an extent that fluid would leave the vascular bed. The cholinesterase might destroy the acetylcholine, with a subsequent vasoconstriction and a redistribution in the body fluids. Thus the fluid returned to the blood under these conditions would remain in the vascular bed, while fluid injected into the blood stream when the vessels were dilated would leave the blood in a very short time. This is very well demonstrated in the experiments of Harkins, Boals, and Chunn (11) and Duncan and Blalock (6), who found that in traumatic shock infusion of plasma causes a greater loss of fluid at the site of trauma. This means that fluid leaks through the capillaries during shock. This situation apparently is alleviated by cholinesterase.

Also cholinesterase may have other functions than just destroying the acetylcholine formed during transmission of the nerve impulse, for cholinesterase is found in tissues where there are no nerves and which, as far as can be determined, are not under nervous control. The red blood cell, rich in cholinesterase, is a good example. One also must not overlook the possibility that the body during shock may form inhibitors of cholinesterase which are not detected in the chemical determination of this enzyme, and that the extra cholinesterase administered may be enough to swing the animal towards a normal state.

#### SUMMARY

1. Surgical shock was produced in 66 anesthetized dogs by excessive hemorrhage or manipulation of the intestines. When permanent shock levels of blood pressure were demonstrated to be present, treatment consisting of either restoration of blood volume or injection of cholinesterase was attempted.

2. The dogs in *hemorrhagic* shock responded well to beef plasma, administered in appropriate volumes, by recovering from shock.

3. The dogs in *traumatic* shock were benefited by plasma only temporarily.

4. When the dogs in *traumatic* shock were given intravenous injections of cholinesterase, the blood pressure nearly always (16 out of 18 dogs) returned to normal and remained there for the duration of the experiment.

Since this work was initiated at the University of Chicago, I wish to thank Drs. A. J. Carlson and A. B. Luckhardt for their guidance and courtesies.

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# THE EFFECT OF ADRENALECTOMY ON THE POTASSIUM OF STIMULATED MUSCLE

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It has been shown that stimulation of skeletal muscle in many animals leads to a loss of potassium from the muscles in exchange for sodium with a gain of sodium chloride and water (Fenn and Cobb, 1936; Fenn et al., 1938; Fenn, 1937). This loss of potassium during muscular activity persists on rats raised on a potassium deficient diet, although a lack of potassium intake will inhibit adequate growth (Heppel, 1940). The following work is an attempt to confirm the results of J. C. Somogyi and Verzar (1941) who were unable to demonstrate a K loss from the skeletal muscles of adrenal-deficient cats, even though the muscles of these animals accomplished the same amount of work as those of normal animals in which a loss of potassium was easily shown.

**METHODS.** Male albino rats, weighing from 80 to 100 grams, were adrenalectomized in one operation using ether as the anesthetic. No vessels needed to be tied and there was rarely more than slight bleeding.

Symptoms of adrenal deficiency usually developed in seven to ten days. The criteria adopted as evidence of insufficiency were hypothermia, anorexia, ataxia, profound inactivity unless probed, and weight loss.

When such signs had manifested themselves, the rats were anesthetized by subcutaneous injection of 1 mgm. per 100 grams body weight of urethane in normal saline (25 per cent solution). Occasionally it was necessary to supplement this with a little ether. The sciatic nerve on one side was exposed and cut in the sciatic notch, after which it was drawn through two silver wire electrodes in a glass tube. The knee on the same side was fixed to a board; the Achilles tendon was freed and attached by means of ligature thread to an isometric lever. The initial tension was set and automatically maintained at 50 grams by means of a pulley arrangement described by Fenn (1938). Stimulation was intermittent, producing 0.4 second of just maximal tetanus alternating with 0.4 second of rest. A thyratron stimulator was used to produce the discharges, and the frequency in all cases was about 58 shocks per second. The cathode was attached to the distal electrode. In all cases the stimulus was maintained for exactly 30 minutes whether or not the muscles continued to contract. All contractions were recorded on a slowly moving kymograph drum.

Immediately after the period of stimulation, the animal was bled to death and the approximate length and maximum diameter of the gastrocnemius muscle group were measured. Also the soleus plantaris muscles were measured and analyzed separately. Since the results obtained with these muscles parallel those of the gastrocnemius group the data are not given. The tissues were freed of fat and nerves, weighed in tared weighing bottles before and after drying

TABLE 1

*Potassium and water changes in stimulated muscles of adrenalectomized and normal control rats*

1	2	3	4	5	6	7	8	9	10
Rat	Wt.	Duration of contraction	Tension time	Average tension	Muscle potassium		Muscle water		
					Resting K	Stim. Δ K	Resting H <sub>2</sub> O	Stim. Δ H <sub>2</sub> O	
Control animals									
	gms.	min.	gm.-min.	gm.	gm./cm <sup>2</sup> .	mM.	mM.	ml.	ml.
C-2	94	30	772	25.7	40.4	53.82	-3.33	350	-13
C-3	100	30	1500	50.0	130.0	52.29	-6.56	333	-101
C-4	100	30	1068	35.6	92.6	53.57	-11.62	340	-68
C-5	100	30	1424	47.4	123.3	47.48	-6.51	338	-76
Av.....	98	30	1191	39.7	96.6	51.79	-7.01	341	-64
Adrenalectomized animals									
10	66	0.68	120	4.0	16.8	54.75	-.13	352	-7
5	68	1.04	145	4.8	17.1	54.34	-.65	331	-5
22	66	1.20	131	4.4	11.3	53.66	-.01	355	-8
13	104	1.9	162	5.4	10.8	51.51	-.95	323	-30
16	98	2.4	178	5.9	9.3	54.16	-1.88	330	-20
20	89	6.9	151	5.0	7.9	55.97	-1.12	349	-32
12	96	30	317	10.6	21.0	52.71	-.40	329	-58
4	120	30	745	24.8	43.8	55.6	-.90	362	-71
19	85	30	250	8.3	13.1	53.8	-1.50	330	-33
3	110	30	410	13.7	27.2	54.7	-1.90	342	-41
11	84	30	461	15.4	27.1	53.4	-3.70	344	-34
2	100	30	337	11.3	22.4	53.8	-4.00	353	-50
Av.....	100	16.2	284	9.5	19.0	54.03	-1.32	337	-34
Normal adult rats (Heppel, 1940).....						47.45	-7.35		-39
Normal rats (Fenn and Cobb, 1936).....						47.3	-6.1	318	-49

Data on individual rat experiments plus averages of results of other authors for purposes of comparison.

Column (1) Rat number.

Column (2) Weight of rat in grams.

Column (3) Minutes of actual muscle contraction (in all cases, stimulation was continued for 30 min.).

Column (4) Average tension in grams for time of actual contraction times minutes of contraction.

Column (5) Average tension in grams.

Column (6) Average tension in grams divided by cross section of muscle in cm<sup>2</sup>.

Column (7) mM of K per 100 grams dry weight of unstimulated muscle.

Column (8) Difference in K content between stimulated and unstimulated muscles, expressed as mM K per 100 grams dry weight.

Column (9) Water content of unstimulated muscle, expressed as cubic centimeters per 100 grams dry weight.

Column (10) Difference between water content of stimulated and unstimulated muscles, expressed as cubic centimeters per 100 grams dry weight.



to constant weight at 98°C., and analyzed in duplicate for potassium by the Shohl-Bennett method as modified by Fenn et al., 1938.

**RESULTS AND DISCUSSION.** The detailed data for the control and adrenalectomized animals are shown in table 1 and the average K and H<sub>2</sub>O changes are compared with similar results of other authors. In the adrenalectomized ani-

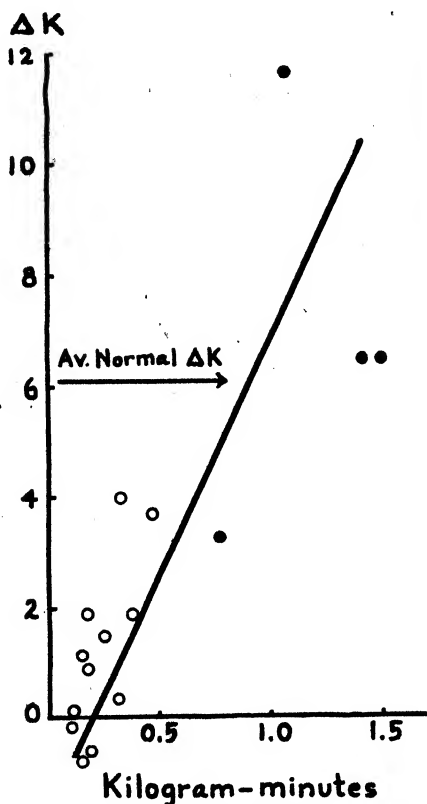


Fig. 1A

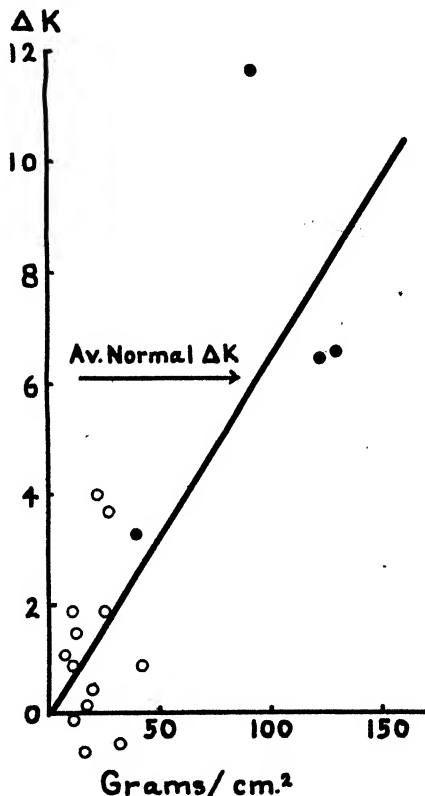


Fig. 1B

Fig. 1. A. Mass plot showing the relation between the K loss from stimulated rat muscles and the tension-time exerted by the muscle. The coefficient of correlation is 0.78.

B. Mass plot showing the relation between the K loss and the average tension per cm.<sup>2</sup> of cross section. The coefficient of correlation is 0.58.

In both graphs each point represents an individual rat. Black dots represent control rats and the clear circles adrenalectomized animals.

mals, the average loss of muscle K was only  $\frac{1}{3}$  of that of the controls ( $-1.32$  as compared to  $-7.01$  mM per 100 grams dry weight). However, the muscles also contracted less than normal as was expected from the work of Haslerud and Ingle (1935). The amount of contraction was estimated from measurements of the tension time (mean tension in grams  $\times$  duration of tetanus in min., table 1, column 4) and the average tension per cm.<sup>2</sup> of cross section (table 1,

column 6), which gave values respectively  $\frac{1}{4}$  and  $\frac{1}{5}$  of the normal. According to these criteria, therefore, the loss of K is correlated with the amount of contraction. This can be seen from figure 1 in which the loss of K in the individual rats is plotted against the corresponding tension-time in gram-minutes and against tension per unit of cross sectional area in grams per cm<sup>2</sup>. The coefficients of correlation for these two lines are 0.78 and 0.58 respectively. Both control (black dots) and adrenalectomized (clear circles) animals are included. The resulting scatter of points is large, but is not inconsistent with the idea that the adrenalectomized rats lost as much K as could have been expected from the small amount of contraction of which the muscles of these animals were capable. The straight lines in figure 1, A and B, were fitted to the points by the method of least squares.

The average H<sub>2</sub>O gain (table 1, column 10) is within the range of the normal. This is to be expected, since Heppel (1940) has shown that the gain in water is maximal after five minutes of stimulation. Table 1 shows that in those rats whose muscles did not contract for five minutes the gain in H<sub>2</sub>O was less than the normal. With this exception, however, the water gain in the adrenalectomized rats was normal, and, indeed, the average gain in water of all the adrenalectomized rats (34 cc.) did not differ significantly from the normal figure which varied from 13 to 101 cc. in 4 animals.

The average K in mM per 100 grams dry weight of the unstimulated muscles of the experimental rats is 54.03 which is higher than the control rat values by 3 to 6 mM per 100 grams. Nevertheless, they do not uphold the very high values obtained in adrenal-deficient rats by Buell and Turner (1941).

#### SUMMARY

Muscles of adrenal-deficient rats were found to lose potassium during indirect electrical stimulation. The loss of potassium was roughly proportional to the product of the tension developed and the time of maintenance of that tension in minutes.

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# THE CONTENTS OF THE STOMACH, SMALL INTESTINE, CECUM AND COLON OF NORMAL AND FASTING RABBITS<sup>1</sup>

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Beaumont's (1) observations on the time of evacuation of carbohydrates, fats and proteins from the stomach of man appeared in 1833, and his results have been confirmed by numerous investigators using various methods. Cannon (2) made roentgen observations on cats and estimated the amount of gastric emptying from the length of the shadows in the intestine. McClure, Reynolds and Schwartz (3) mixed barium sulphate with foods and observed the passage fluoroscopically in man.

The emptying time of the gastrointestinal contents of rabbits after a four day fast was observed by Swirski (4) who weighed the contents. Using a similar technique, Carmichael (5) reported that a large dose of oleoresin of capsicum did not markedly affect the emptying of the rabbit's stomach within 24 hours when the animal was not muzzled.

The study reported here was planned to determine the amounts of the contents of the stomach, small intestine, cecum and colon of rabbits on a normal diet and of those that had fasted with and without a muzzle for different lengths of time.

**EXPERIMENTAL.** All animals were given a diet of alfalfa hay, oats and water at least a week before they were used. Green foods were ruled out of the diet since these foods would have a tendency to cause a greater variation in the contents of the gastrointestinal tract because of the variability in the type and amount of such foods available to the animal at different times. Three series of young animals were run: 1, normal control animals—weight range 1183 to 2174 grams; 2, animals that fasted without muzzles—weight range 1045 to 1806 grams, and 3, animals that fasted with muzzles to prevent coprophagy which occurs normally in caged animals—weight range 1215 to 1885 grams. Water was present at all times for all animals in each series. The three series of experiments included 22 normal controls, 7 animals that fasted without muzzles and 35 animals that fasted with muzzles.

The contents of each of the four sections of the G.I.T., i.e., stomach, small intestine, cecum and colon, were removed as completely as possible, weighed and dried to constant weight at 100°C. Since it was impossible to remove all of the contents from the sections by mechanical means, each section was washed out with distilled water and this water was evaporated and the residue also was dried at 100°C. The weight of the dry residue from the wash water was added

<sup>1</sup> Aided by a research grant from the University of Alabama.

to that of the respective contents which had been removed by mechanical means. For wet weight values of the contents that were washed off each of the four sections the dried residue was calculated for water content on the basis of the material that was removed mechanically, and that value was added to the wet weight of the respective contents. Table 1 presents the results of both the normal and fasting animals.

TABLE 1

*Contents of the G.I.T. of normal rabbits and of rabbits that had fasted either without a muzzle or with a muzzle*

NO. OF ANIMALS	LENGTH OF FAST	WT. OR AVERAGE WT.	AVERAGE WET WEIGHT CONTENTS					AVERAGE DRY WEIGHT CONTENTS				
			Stom-ach	S. int.	Cecum	Colon	Total	Stom-ach	S. int.	Cecum	Colon	Total
Controls												
	days	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
10		1337	88.95	24.67	80.69	17.57	211.88	19.93	2.47	18.87	4.76	46.03
6		1731	101.38	24.98	97.53	24.39	248.28	21.88	2.02	20.95	6.70	51.55
6		2003	121.82	23.31	100.62	20.77	266.52	27.21	2.5	21.53	6.26	57.50
Fasting without muzzle												
1	1	1503	74.01	24.80	67.99	7.72	174.52	15.77	2.83	12.83	1.72	33.15
1	2	1045	59.22	11.54	70.02	12.28	153.06	11.70	0.87	14.80	3.24	30.61
1	3	1175	27.47	24.43	71.76	7.08	130.73	5.92	2.56	16.18	1.90	26.56
1	4	1195	16.35	18.26	52.20	12.82	99.61	3.13	2.31	13.13	3.74	22.31
1	5	1187	14.73	15.46	55.73	9.07	94.98	2.84	1.22	13.90	2.46	20.41
1	6	1655	48.67	10.41	61.46	12.83	133.36	9.93	0.93	15.40	3.83	30.09
1	7	1806	66.11	8.59	53.27	11.37	139.34	15.80	0.85	13.31	3.81	33.76
Fasting with muzzle												
6	1	1584	31.60	19.95	79.99	8.57	140.11	3.64	1.58	15.42	2.43	23.07
6	2	1442	12.24	15.76	50.54	4.79	83.33	0.78	1.37	9.89	1.31	13.35
3	3	1573	14.94	24.01	39.62	1.97	80.54	0.60	1.67	6.40	0.48	9.15
3	4	1479	20.60	17.02	25.55	2.69	66.46	1.32	1.25	4.78	0.55	7.90
3	5	1535	15.35	15.71	26.83	2.00	60.89	1.45	1.47	4.74	0.62	8.28
3	6	1444	10.33	15.77	27.00	1.14	54.24	0.52	1.19	6.56	0.36	8.63
3	7	1392	5.81	14.28	29.10	1.71	50.90	0.19	1.22	7.18	0.53	9.12
2	8	1357	12.68	9.76	32.37	1.42	56.23	0.48	0.79	7.57	0.19	9.38
2	9	1484	7.83	11.28	28.85	2.17	50.13	0.63	1.09	5.26	0.40	7.30
2	10	1561	7.10	8.95	23.19	1.35	40.59	0.50	0.69	4.83	0.28	6.30
2	11	1587	15.60	12.57	14.34	1.93	44.44	0.44	0.98	3.45	0.44	5.31

In the normal control rabbits there was a gradual increase in the contents of the stomach and the cecum as the weight of the animal increased, while the contents of the small intestine and the colon were quite variable. However, both the wet and the dry weights of the G.I.T. contents as a whole increase with the heavier animals.

The results on the animals that were allowed to fast without muzzles illustrate

how the wet contents of the G.I.T. was maintained at a higher level than the corresponding contents from animals that fasted with a muzzle for the same length of time. This also holds if we consider the total weight of the dry contents of the G.I.T. In other words, we have what has been termed as a circulation of the feces in rabbits fasting without a muzzle as well as in normal animals.

The dry contents of the gastrointestinal tract of the six muzzled animals decreased about 50 per cent during the first 24 hours of the fast when we compare the values with those of the normal controls. After 48 hours' fasting, the dry contents showed a decrease of about 50 per cent from the 24 hour value. The decrease in dry contents during the next 24 hours was less marked, but it continued fairly gradually until on the eleventh day of the fast when the residue was about equivalent to 10 per cent of the values of the normal controls.

The wet stomach contents varied over a wide range because some of the animals took water just previously to the time that they were killed. The total weight of the stomach contents has been given here, but actually the liquid part was weighed separately in many instances. Sometimes the solid material consisted only of a mat of hair, and in other cases there was a small amount of food particles on the mucosa of the stomach and in the mat of hair. The dry weights of the stomach contents were more constant than the wet weights, since the latter were often altered by the recent drinking of water.

It has been stated that in most vertebrates the stomach functions both as a digestive organ and as a reservoir. This seems to be true also in the case of the cecum since in normal animals it contains almost as much material as the stomach. In the case of the animals that had fasted with a muzzle 24 to 240 hours the average weights of the contents of the ceca were consistently greater than those of the stomachs of the same group, even though the animals may have drunk water just before being killed.

The physiology of secretion exhibited in the small intestine of fasting animals is of especial interest since the secretion of the glands continued after the stomach was free from visible solid foods. The contents of the small intestine of the control animals was a viscous mucus which incorporated small amounts of visible food particles. The contents of the small intestine of the fasted animals with a muzzle was less than that in the controls, and following a fast of two or more days there were fewer visible food particles than in the controls, but the viscosity and general appearance of the contents were about the same as that for the controls.

The maximum and minimum weights of the secretion of the small intestine for individual animals of each series of experiments are given for both the wet and dry contents (table 2). The average values for both wet and dry contents are given for each series. The fasting animals had about 65 per cent as much wet contents as the controls but those that fasted with a muzzle had only about 55 per cent as much dry contents as the controls.

The maximum and minimum per cents of dry contents for individual animals of each series are also presented and these values are of about the same order in all three series. These results seem to indicate that the small intestine of either normal or fasting rabbits yields a secretion that has about the same per cent of

**solid material.** However, the average per cent of dry matter is slightly less in the case of the animals that fasted with a muzzle than it was in the controls.

Swirski (4) used a diet of oats, clover and bread for his rabbits, and his animals that fasted with a muzzle had much less dry G.I.T. contents from the 4th day to the 8th day than our animals for the same period. His values were lower on the 4th day of the fast than our values were on the 10th day. This may have been due to the type of diet that he used. The diet that we employed could be used in any season in most countries.

We believe that rabbits that have fasted with a muzzle for 24 to 48 hours would give much more constant results following oral administration of drugs than well fed animals, since the latter normally contain such large quantities of fresh food in addition to a good quantity of feces.

TABLE 2

*Studies on the contents of the secretion of the small intestine of normal and fasted animals*

SERIES	NO. OF ANIMALS	WET CONTENTS OF SMALL INTESTINE			DRY CONTENTS OF SMALL INTESTINE					
		Individual animals		Average wt. for series	Individual animals		Average wt. for series	Per cent for individual animals		Average per cent for series
		Max.	Min.		Max.	Min.		Max.	Min.	
		gm.	gm.		gm.	gm.		gm.	gm.	
Controls.....	22	39.78	8.87	24.38	3.9	1.04	2.35	13.93	5.9	9.65
Fasting without muzzle.....	7	24.80	8.59	16.07	2.83	0.85	1.65	12.65	7.24	10.26
Fasting with muzzle.....	35	29.17	6.5	16.16	2.23	0.61	1.30	13.37	5.13	8.03

## SUMMARY

1. Twenty-two normal rabbits were sacrificed and the contents of their stomachs, small intestines, ceca and colons were removed, weighed and dried to constant weight at 100°C.

2. Seven rabbits were allowed to fast without muzzles and the contents of their gastrointestinal tracts were weighed and dried at 100°C.

3. Thirty-five rabbits were fasted with muzzles from one to eleven days and the contents of their G.I.T. were weighed and dried at 100°C.

4. The stomach contents dropped to about 50 per cent of the normal within 24 hours after the fast started. Within a few days, the stomach contents consisted either of a very small quantity of food particles, water and a mat of hair, or only the latter two.

5. The contents of the small intestine decreased as the fast continued, but the viscosity and general appearance of the contents seemed to be fairly constant except that the suspended particles became less as the fast lengthened. The dry residue of the intestinal contents from the animals that fasted with a muzzle was about the same per cent of the total wet weights as the residues were for the control animals.

6. The cecum continued to act as a reservoir for food materials after the stomach had been emptied of visible solid foods.

7. The contents of the colon decreased rather rapidly for the first two days of a fast with a muzzle. The dry content of the colon was fairly constant from the third to the eleventh day of the fast.

8. In studying the physiological effects in the rabbit of orally administered drugs, more constant results should be obtained if the animal is allowed to fast with a muzzle 24 to 48 hours before the drug is given.

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# THE DEHYDRATING EFFECT OF CONTINUOUSLY ADMINISTERED WATER<sup>1</sup>

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Gamble (1937), Stewart and Rourke (1942) and others have shown that the quantity of water lost in a diuresis due to water excess may be greater than the quantity taken in. Generally the lost excess is believed to be related to the amount of salt which escapes from the body during water diuresis. The present work is a study of this dehydrating effect in man.

**PROCEDURES.** Ten young subjects (nine men and one woman) whose average weight was 67 kgm. were used. One to one and a half hours post-prandially the bladder was emptied, the urine discarded, and a fixed amount of water was drunk every ten minutes. Every half-hour thereafter urine was collected. An aliquot was analysed for chloride and its concentration expressed as mgm./cc. of NaCl. With the subject at rest or engaged in minimal activity, the drinking continued for periods of either 3 or 7 hours, at rates ranging from 20 to 200 cc. per ten minute period (expressed as 2 to 20 cc./min., respectively). The conditions were not conducive to sweating. There was no ingestion other than water.

**RESULTS.** Figure 1 shows the average of the ratios of urinary output rate to intake rate during 7 hours. Intake rates varied from 6 to 10 cc./min. from experiment to experiment. Even in the presence of normal insensible loss, the urinary output was sustained at a greater level than the intake. Figure 2 indicates for the same experiments, the average urinary chloride concentration and the average excretion rate of chloride, as they varied with time. A steady state seems to have been reached from about the third hour. In figure 3 concentration and excretion rate are shown as a function of the rate of intake. By the 7th hour the intakes from 6 to 10 cc./min. had the same effect on the final urinary chloride concentration; and for this reason these experiments were combined for figure 1.

Figure 4 shows the net load of water at the 7th hour had no clear relation to the rate of intake. The loads were predominantly negative and in more prolonged experiments they should become more decisively negative if the output were maintained at a higher level than the intake.

**DISCUSSION.** The demonstrated dehydrating effect of water may be examined quantitatively. Prior to this the concept of threshold concentrations requires definition. Two distinct viewpoints are held.

The first has been discussed by Cushny (1926), Aitken (1929), MacKay and MacKay (1936), and by Hare, Hare and Phillips (1943). It holds that if a

<sup>1</sup> This work was aided by a grant from the Winthrop Chemical Co.



threshold concentration is to be established there should be a plasma concentration of the substance in question above which the substance will appear frankly

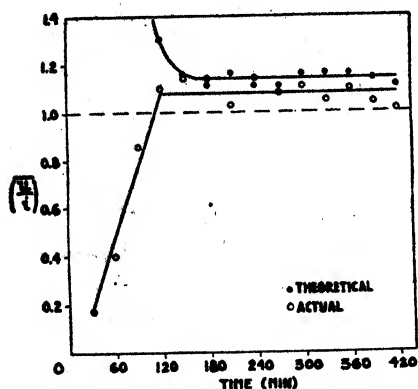


Fig. 1

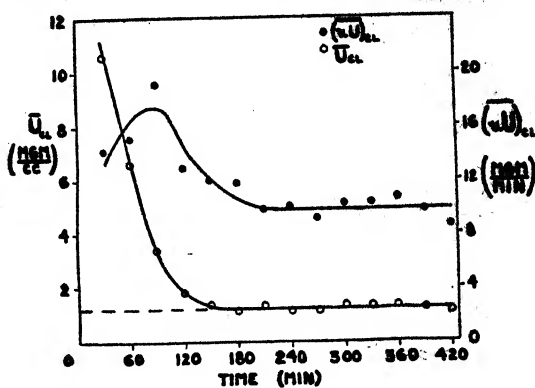


Fig. 2

Fig. 1. Average of actual ratios of urine output rate,  $u$ , to water intake rate,  $i$ , obtained in 3 and 7 hour experiments combined. Average of 26 experiments for each point for first three hours; 12 experiments for other points. Intake rates include 6, 7, 8, 9 and 10 cc./min. The theoretical values (black dots) from equation (3) are corrected by assuming an insensible loss,  $w$ , of 0.7 cc./min. Theoretically the total fluid output should be 25 per cent greater than the intake when  $U_{Cl} = 1.2$ .

Fig. 2. Average urinary Cl concentration ( $\bar{U}_{Cl}$ ) and average excretion rate of Cl ( $(\bar{u}\bar{U})_{Cl}$ ) varying with time. Averages for first three hours are from 22 to 24 experiments; 12 experiments for other points. Urinary Cl concentration reaches a minimum of 1.2 mgm./cc. by the third hour.

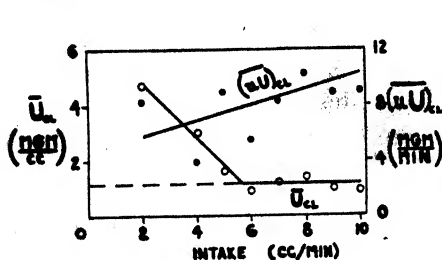


Fig. 3

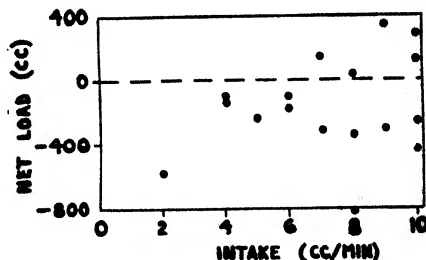


Fig. 4

Fig. 3. Average urinary Cl concentration ( $\bar{U}_{Cl}$ ) and average excretion rate of Cl ( $(\bar{u}\bar{U})_{Cl}$ ) at 7th hour, related to intake rate. Values at intake rates of 2, 4, 5, 6, 7, 8, 9 and 10 cc./min. are averaged from 1, 2, 1, 2, 2, 3, 2 and 4 experiments respectively.

Fig. 4. The net or sensible load was obtained when 294 was subtracted from the difference between fluid intake and output at the end of the 7th hour, to correct for the assumed insensible loss of 0.7 cc./min., i.e.,  $0.7 (420) = 294$ .

in the urine, and below which the urinary excretion of the substance is sharply reduced.

The studies of Smith (1943) and his associates, and others, indicated that this threshold concentration may not be a critical value, but may be rather, as in the

case of glucose, a range, in the lower parts of which the reabsorptive capacity of certain tubules becomes saturated before that of others, resulting in the initial "spilling" of glucose. Since the difference between the rate of loading of the tubules with glucose from the glomeruli, and the actual rate of excretion of glucose by the kidney is taken to represent the rate of reabsorption by the tubules, a maximal rate of reabsorptive transfer of glucose by the tubules, which is designated  $T_m$ , can be correlated with a threshold concentration. Smith (1937), and Hare, Hare and Phillips (1943) observe that chloride has no ascertainable  $T_m$ . The tubules when presented with increased loads of chloride not only reabsorb more chloride but they "spill" more into the urine. And the absence of a  $T_m$  for chloride has been correlated with the absence of a threshold. At widely varying plasma levels, chloride may frankly be found in the urine.

The second concept of threshold concentration has been discussed by Rehberg (1926), Conway and Kane (1929), Wolf (1943), and Dillon (1943). The latter author stresses the difference between these two concepts as follows. "Some confusion has crept in between the threshold of appearance and the much more important point of plasma concentration, above which chloride is concentrated in the urine at all rates of urine secretion." The two concepts can be distinguished by specific terms such as *threshold of appearance* for the former, and *threshold of retention* for the latter. The latter has, for some substances such as chloride, the same value as the normal plasma concentration. With minimal extra-renal water loss, the threshold of retention may be defined in terms of the relative urinary retention of chloride to water. A man taking a solution whose concentration is super-threshold, excretes a urine of super-threshold concentration. A sub-threshold solution results in the excretion of urine of sub-threshold concentration. The threshold of retention, unlike the threshold of appearance, is a critical value easily determined in dog (Wolf, 1943) or man. In the dog it is 6 mgm./cc. for chloride. Infusion of a solution of 4 mgm./cc. chloride is followed by the formation of a still more dilute urine; infusion of a solution of 8 mgm./cc. chloride is followed by the excretion of a more concentrated urine; but infusion of a solution of 6 mgm./cc. chloride will produce urine of equal chloride concentration.

*The theory of the steady state.* A liter of water loaded on a man makes his water load positive. When the liter is excreted and he regains his original water content, his salt load is negative due to the loss of salt colligated with water. If the kidneys are to keep the plasma concentration constant, one method of succeeding would be to excrete additional water and reconcentrate the diluted plasma. Even the removal into the urine of salt solution, provided it be more dilute than the plasma, would accomplish this end.

By the third hour in these experiments, the urinary fluid output settled to an essentially steady value, greater than the intake. One hypothesizes that during a steady state of water intake, the excess of water loss above intake should be related to the quantity of salt lost. In a steady state the rate of excretion of salt per excess of fluid output over intake should be equal to the normal plasma concentration of chloride (i.e., equal to the threshold of retention) since only by

removing from plasma a solution of concentration equal to that of the plasma itself, can we leave the plasma with the same concentration. The threshold of retention in both man and dog is approximately 6 mgm./cc. of chloride so that neglecting extra-renal water loss,

$$\frac{uU_{Cl}}{u-i} = A_r = 6 \quad (1)$$

where  $u$  and  $i$  are the number of cc./min. of urine flow and of intake, respectively;  $U$  is the concentration of chloride in the urine expressed in mgm./cc.; and  $A_r$  is the threshold of retention in mgm./cc. From (1) by transposition we get

$$\frac{u}{i} = \frac{6}{6 - U_{Cl}} = \frac{A_r}{A_r - U_{Cl}} \quad (2)^1$$

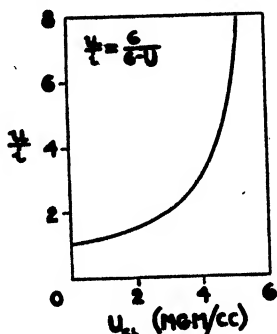


Fig. 5. The theoretical ratio of fluid output to intake rate for given "leakage" concentrations of urinary Cl.

From (2) if we know the concentration of urinary chloride we can calculate the ratio of output to intake when the steady state has been attained. By using the average values for urinary chloride from figure 2, the theoretical  $u/i$  values were obtained for figure 1. The theoretical values, corrected for insensible loss, show noteworthy agreement with the actual values after the third hour. The actual urinary fluid output is about 8 per cent greater than the intake and the total output including insensible loss, is possibly 15 per cent to 25 per cent greater. These experiments add further support to the thesis that the kidneys regulate concentration first; the maintenance of volume is secondary.

From equation (2) it is apparent that if no chloride "leaked" (i.e.,  $U_{Cl} = 0$ ), then  $u/i$  would be unity. The greater the leakage concentration, the greater the  $u/i$  ratio would be if the kidney is to maintain chloride homeostasis (fig. 5).

<sup>1</sup> To correct for insensible loss,  $w$ , in cc./min., relate the excretion of chloride to  $(u + w - i)$  and obtain

$$\frac{u}{i} = \frac{6 \left( 1 - \frac{w}{i} \right)}{6 - U_{Cl}} \quad (3)$$

The use of the steady state equation (1) or (2) can be qualified. "Steady state" does not imply "equilibrium". Intake may be steady but the output may or may not be so and in any case may be different from the intake. Presumably physiological regulations are steady. The loads are not. For very short durations, or for minimal rates of intake, the physiological state may not attain a steady state. The equation of steady state is useless for very high rates of intake (over 10 cc./min. in a few experiments) as the water output may not only fail to exceed the intake but may drop below it, the water load increasing rapidly. The equation is adapted to computation where average values of diuretic urinary chloride are used, since small individual variations in  $U$  may have large effects on a calculated  $u/i$  value. The equation can only be used properly for those substances which have a threshold of retention, i.e., whose plasma levels can be raised or lowered by renal regulation alone. Presumably chloride and sodium fit this category, perhaps potassium.<sup>3</sup>

#### CONCLUSIONS

1. A steady state of water intake under the conditions of these experiments on man results in a total output of fluid, larger than the intake, and if continued, leads to the production of negative water loads. The urinary output alone was 8 per cent greater than the intake rate.

2. The ratio of rate of chloride excretion to rate of excess water excretion is equal to the normal plasma concentration in the steady state, and the equation of steady state is derived.

3. The threshold of appearance and the threshold of retention are defined and are illustrated for chloride.

4. In renal excretion, the regulation of concentration of plasma chloride takes precedence over the regulation of body volume, when water is drunk.

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<sup>3</sup> Under suitable conditions it is possible to estimate either the insensible loss or the threshold from the other factors as

$$w = i - u + \frac{uU_{Cl}}{A_T} \quad (4)$$

and

$$A_T = \frac{uU}{u - i + w} \quad (5)$$

# THE RETENTION AND EXCRETION OF CONTINUOUSLY ADMINISTERED SALT SOLUTIONS<sup>1</sup>

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The accepted interpretation of the retention of certain administered saline solutions is inadequate in several particulars. First, the belief that physiological saline resembles so closely the normal plasma that it provokes no extra activity is untenable if we examine the activity of the kidney a sufficiently long time after the onset of infusion when equilibrium is approached. Stewart and Rourke (1942) show how continuous infusions of 0.9 per cent NaCl after several days become markedly diuretic and the initial increasing tide of water load is turned; output can then exceed intake rates. And it is demonstrated (Wolf, 1943) that the kidneys react just as quickly and critically to physiological saline as to water in modifying urinary concentration.

Second, it is unlikely that solutions such as 0.9 per cent to 1.3 per cent NaCl are retained in the tissues by virtue of the "osmotic" binding of the water to the salt since Wolf (1943) showed that infusions ranging from 0.6 per cent to 1.7 per cent NaCl result in the more rapid excretion of chloride than of water. Such saline is, at least with respect to chloride, super-threshold, and is diluted in the body as a result of the excretion of urine more highly concentrated than the intake fluid.

Third, the concept of the pre-renal deviation of saline into the tissues and away from the kidneys is an *a posteriori* argument, unsuitable in view of all facts.

Fourth, water retention cannot always be based on the activity of Na or some other particular ion. NaCl and NaHCO<sub>3</sub> may be edema forming (under pre-equilibrium conditions or for certain intake concentrations) at some times but not at others (Stewart and Rourke, 1942). It is easy to point to substances like Na<sub>2</sub>SO<sub>4</sub> or KCl which so readily leave the body with water, if we wish to absolve of guilt either the Na or the Cl as an individual. The present experiments grew out of previous work (Wolf, 1943) in an attempt to extend to man the information obtained on the relative retention of salt to water in the dog.

**PROCEDURES.** Steady rates of ingestion of salt solutions of various concentrations were maintained in experiments of 3 hours' duration in 3 subjects at rest or engaged in minimal activity. Every 10 minutes 100 cc. of fluid was drunk (expressed as 10 cc./min.). Every half hour urine was collected, except for the contents of the bladder which were discarded at the beginning of the experiment. An aliquot was analysed for chloride and its concentration expressed in milligrams of NaCl per cubic centimeter. The conditions were not conducive to sweating. There was no ingestion other than the experimental fluid.

<sup>1</sup> This work was aided by a grant from the Winthrop Chemical Co.

**RESULTS.** Figure 1 shows the actual average ratio of rate of urinary fluid output to intake at the end of the third hour in man, for different concentrations of chloride in the intake fluid. With concentrations over 10 mgm./cc. of NaCl, diarrhea tended to set in by the end of 3 hours and large volumes of fluid were in the gut in these cases. Figure 2 for the same experiments shows the average urinary concentration at the third hour related to intake concentration. Figure 3 shows some ratios of fluid output to intake in the dog resulting from intravenous administration of fluid (computed from Wolf, 1943).

**DISCUSSION.** The theory and equation of the steady state (Wolf, 1945) are probably special aspects of a more general theory. They described the behavior of administered water which is the case of an intake concentration of

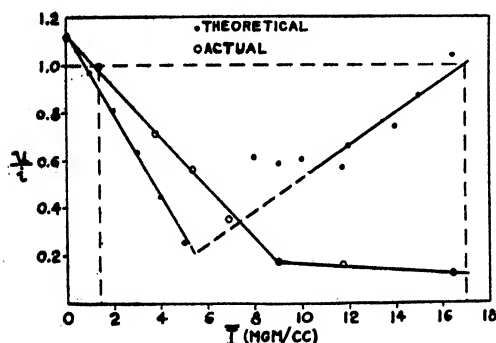


Fig. 1

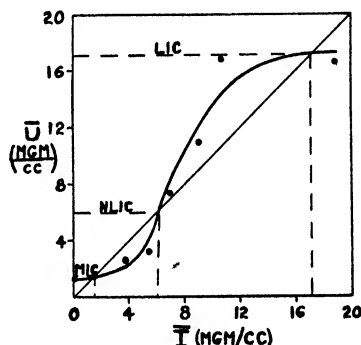


Fig. 2

Fig. 1. The points representing actual average  $u/i$  (urinary output rate,  $u$ , divided by intake rate,  $i$ ) are derived from 49 experiments in man over the entire range of intake concentrations (to  $I = 19$  mgm./cc.). The theoretical  $u/i$  values (equation (3)), based on the average urinary concentrations from figure 2, are corrected for an assumed insensible loss,  $w$ , of 0.7 cc./min. The value  $(I - 6)/(U - 6)$  from the equation cannot be determined as  $I$  and  $U$  approach 6, so interrupted lines are extrapolated on the theoretical curve to correspond to figure 3. There is no other justification for such extrapolation.

Fig. 2. Average urinary chloride concentration ( $\bar{U}_{Cl}$ ) at the end of the third hour. The points represent averages of a total of 49 experiments in man. When  $\bar{U} = I$ , the solutions are isorrheic, at the minimal (MIC), non-limiting (NLIC), and limiting (LIC) isorrheic concentrations.

zero. If the kidneys are to maintain plasma chloride concentration during a steady state of intake of chloride as well as water, the ratio of chloride excreted in excess of chloride taken in, per fluid excreted over intake, should equal the normal plasma concentration or the threshold of retention, i.e., uncorrected for insensible loss

$$\frac{uU - iI}{u - i} = A_r = 6 = \frac{iI - uU}{i - u} \quad (1)$$

where  $u$  and  $i$  are respectively the cc./min. of urinary output and of intake;  $U$  and  $I$  are respectively the concentrations of urine and of intake fluid in mgm.

NaCl/cc., and  $A_r$  is the threshold of retention in mgm./cc. By transposition of equation (1)

$$\frac{u}{i} = \frac{6 - I}{6 - U} = \frac{I - 6}{U - 6} = \frac{I - A_r}{U - A_r} \quad (2)$$

From equation (2), whenever a steady state is obtained, the ratio of fluid output to intake at a given time should depend on the concentration of the intake and the urinary concentration at that given time. Without correction for insensible loss,  $u/i$  will attain unity when  $I$  is 1.4 mgm./cc. since  $U$  will become equal to the same value as  $I$  (fig. 2).<sup>2</sup>

Figure 2 shows two other values of  $I$  which are critical in that they are the only other ones which may be administered without resulting in the relative retention of chloride to water or water to chloride, since the urinary concentration quickly equalizes to that of the intake. In the dog, 6 and 17 mgm./cc. were called *isorrheic concentrations* by Wolf (1943), the former, the non-limiting isorrheic concentration (NLIC) or threshold, and the latter, the limiting isorrheic concentration (LIC). In man these values are here determined to be approximately the same as in the dog. In addition, a minimal isorrheic concentration (MIC) of approximately 1.4 mgm./cc. was determined, the lowest concentration of fluid which can be administered without leading to relative retention of water to salt or salt to water from the intake fluid. Coon, Noojin and Pfeiffer (1941) from a different type of experiment, estimated the latter concentration to be about 2 mgm./cc. Actually any concentration of intake between the MIC and the LIC should result in only temporary retention of salt or water relative to the other. Stewart and Rourke (1942) proved it for 0.9 per cent NaCl.

Figure 4 shows another way of determining isorrheic concentrations in man by the use of the velocity constant of excretion,  $\gamma$ , i.e., the ratio of rate of excretion (mgm. or cc./min.) to load (mgm. or cc.) retained at a given time. When the ratio of the velocity constant of water to that of chloride is unity, it means that the rate of excretion of water is relatively equal to the rate of excretion of chloride, or that their relative retentions are equal. However, in experiments of short duration, the method of figure 2 gives more accurate results.

Figure 2 shows that in the range of 6 to 17 mgm./cc. of intake concentrations, urinary chloride exceeds intake chloride. From equation (2) we see that so long as that is true,  $u/i$  will be less than 1. Chloride is being thrown away relative to water to transform the administered and retained fluid to the threshold (normal plasma) concentration. Water, in other words, is being retained relative to chloride to the same end and the apparent diuretic activity of such solutions (particularly in the range 6 to 12 mgm./cc. for intravenous solutions) is initially very small. However, given time, the retained fluid can ultimately be transformed to one of normal plasma concentration, the need for relative re-

<sup>2</sup> Correction for insensible loss,  $w$ , in cc./min. is given by

$$\frac{u}{i} = \frac{I - A_r}{U - A_r} + \frac{A_r}{U - A_r} \cdot \frac{w}{i} \quad (3)$$

tention of water abates, the urinary chloride falls to the intake concentration (Stewart and Rourke, 1942), and the output and intake rates approach equality.

In figure 3, for the dog, the theoretical  $u/i$  values calculated from the general steady state equation and corrected for the measured insensible loss, agree roughly with the actual values through the range  $I = 0$  to  $I = 17$ . There is no theoretical justification for the equation at values of  $I$  greater than the limiting isorrheic concentration. However, in figure 1, where the intake was oral and not intravenous, the deviation of theoretical and actual values increases as  $I$  becomes larger, although the theoretical curve resembles that for the intravenous data of the dog. Perhaps the greatest part of the discrepancy in figure 1 is a function of the aberrations of absorption, which were especially marked by the

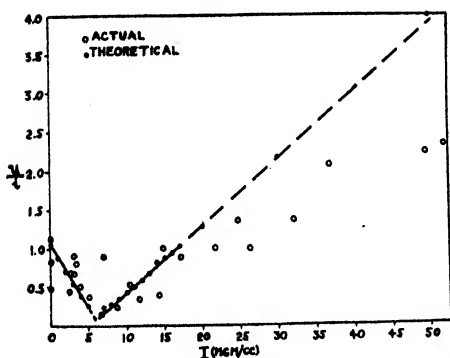


Fig. 3

Fig. 3. Ratio of fluid output rate,  $u$ , to intake rate,  $i$ , with intravenous infusion in the dog. Data of Wolf (1943). Theoretical values are corrected for the known insensible loss. Each point represents one experiment. Intake rate was 2.3 cc./min. There is no theoretical justification for the extrapolation above  $I = 17$ .

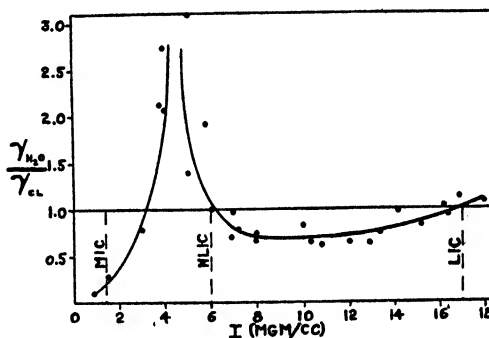


Fig. 4

Fig. 4. The ratio of velocity constants of water and chloride,  $\frac{\gamma_{H_2O}}{\gamma_{Cl}}$ , in relation to intake concentrations,  $I$ , at the third hour in man. The determination of the isorrheic concentrations by this method is not so accurate with experiments of such short duration as these, as compared with the method of figure 2. Thus given more time, the points, particularly at  $I = 3.0$  and  $I = 1.5$  could be expected to shift, moving the MIC toward 1.4 mgm./cc. Uncorrected for insensible loss.

diarrhea following ingestions of solutions stronger than 10 mgm./cc. Coon, Noojin and Pfeiffer (1941) report that ingestion of 4 liters of even 5 mgm. NaCl/cc. in 90 minutes caused diarrhea. This is evidence of absorption characteristics of the gut even at lower intake concentrations which lead to differences between the consequences of oral and intravenous administration. Intravenous studies in man should clarify the nature of the discrepancy between theoretical and actual values.

In man, with negligible extra-renal water loss, the LIC of 17 mgm./cc. is the highest concentration of administered fluid which can be tolerated without relative retention of chloride to water, and is thus a critical physiological value. It differs widely from the "maximal" urinary concentration (where chloride is



retained relative to water) of the order of 21 mgm./cc. found by Adolph (1923). The LIC is useful in analysis of the physiological difficulties following the drinking of strongly hypertonic solutions like sea-water, but it should be remembered that such ingestions create more than renal problems, since the gut deflects fluid in diarrhea.

*The leakage of chloride.* From equation (2), for water drinking, when  $I$  is zero and  $U$  is 1.2 mgm./cc. in the steady state, then with  $A_r$  equal to 6 mgm./cc., we find that  $u/i$  is 1.25. The latter might be regarded in a sense as a constant which should satisfy the equation for other thresholds than chloride. Thus for water drinking

$$1.25 = \frac{A_r - 0}{A_r - U} = \frac{6}{6 - 1.2} \quad (4)$$

and

$$U = 0.2A_r \quad (5)$$

This implies that the "leakage" concentration of any substance handled like chloride by the kidney with respect to regulation of plasma concentration should amount to  $\frac{1}{5}$  of the normal plasma concentration; or that the threshold of retention is 5 times the concentration found in steady state diuretic urine. However, for any given individual, the  $u/i$  may not be 1.25,  $U$  may not be 1.2 (some of the present experiments showed individual values of 0.4), and indeed, the formula even when corrected for extra-renal loss has not been soundly established.

*The variable threshold.* From equation (1) we can compute the threshold of retention especially when the intake concentration is zero. If such a method were used rather than the method of velocity constants, we should find individual determinations varying widely from one time to another in the course of a single experiment. Some of this is systematic and accidental error. But it is not amiss to regard the threshold as a fluctuating value deviating from a mean; there is little reason to believe that a threshold is any more constant than any other physiological variable. The apparent constancy of the normal plasma concentration which is synonymous with the mean threshold is perhaps only a manifestation of the inertia of the ponderous electrolyte system which is unable to respond to the caprices of the oscillating threshold. Dillon (1943) used the concept of a variable threshold of chloride in the interpretation of the diuretic activity of mercury ions, pituitrin,  $\text{Na}_2\text{SO}_4$ , etc. The effect of these substances on the threshold of chloride is taken by him as a basis of their action.

*The retention of water from administered saline solutions.* The administration of physiological saline is rarely continued (or advisable) for sufficient duration to attain isorrheic equilibrium. For solutions of  $\text{NaCl}$  in the range from 0.6 per cent to 1.2 per cent particularly, the rate of excretion of chloride per unit load is initially very small. However small this velocity constant may be, the kidneys excrete water per unit load of water at a still slower rate (i.e., to retain it

relative to chloride) so as to transform the retained chloride to 0.6 per cent. Thus salines in the physiological range initially have the lowest excretion rates of water per unit load of fluid, and are the most edema-forming per unit volume administered. Solutions much less concentrated have higher velocity constants for water and can in many instances relieve dehydration with less risk of overloading body fluid compartments, when kidney activity is unimpaired. Solutions stronger than 0.2 per cent are unlikely to deplete the body of chloride or sodium; and solutions weaker than 0.4 per cent can be fortified osmotically with glucose for intravenous infusion.

Solutions of concentration less than the minimal isorrheic of 1.4 mgm./cc. tend to remove from the body an excess of fluid whose virtual concentration is 6 mgm./cc. In this light, the recommendations of Schroeder (1941) and Schemm (1944) on restricting salt alone rather than both salt and water for the relief of cardiac edema, appear physiologically rational.

*Steady state, isorrhea, equilibrium.* The "steady state" refers to constancy of intake rate which may be imposed as accurately as we please. Its significance may be limited to those rates of intake in which physiological adjustments can be made to enable the organism to get along with minimal disturbances of body fluid concentrations. When the concentration of such intake permits of no relative retention of solute to water, the concentrations of intake and of urine are isorrheic. Isorrheic solutions in steady intake ultimately reach equilibrium or equality of intake rate and output rate of solute and water; characteristically, concentration equality is established first. Solutions other than the MIC, NLIC, and LIC, except those of lower concentration than the MIC or higher than the LIC, will ultimately attain isorrhea but probably reach equality (intake-output) of concentrations and rates of exchange simultaneously.

#### CONCLUSIONS

1. The threshold of retention of chloride in man (expressed as NaCl) is approximately 6 mgm./cc. Two other critical concentrations were determined at which neither chloride nor water is retained relative to the other from administered solutions. The lower, the minimal isorrheic concentration, is 1.4 mgm./cc. The higher, the limiting isorrheic concentration is 17 mgm./cc.

2. The initial fluid retention following administration of physiological and slightly stronger saline solutions is shown to be primarily a retention of water and not of salt.

3. For intravenous infusions of NaCl solutions, the ratio of fluid output rate to intake rate may be estimated from a theoretically derived equation of steady state.

4. For chloride and probably certain other substances there is reason to believe that the "leakage" concentration in urine forming under steady state water intake may be approximately  $\frac{1}{3}$  of its normal plasma concentration (uncorrected for extra-renal loss).

5. The threshold of retention of these substances may not be a fixed value even in the normal state, but may fluctuate about a mean.

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# THE EFFECT OF HEMORRHAGE ON THE LACTATE/PYRUVATE RATIO AND ARTERIAL-VENOUS DIFFERENCES IN GLUCOSE AND LACTATE <sup>1, 2</sup>

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It has been recognized for many years that hemorrhage causes changes in carbohydrate metabolism. Recently, Russel, Long, and Engel (1944) reported that hemorrhage hastened the fall in the blood glucose level of eviscerated starved rats. The authors concluded that hemorrhage produced an increased peripheral utilization of glucose. Govier (1943) found that blood loss in the dog is followed by an increase in the lactate and keto acids. This author associated the increase in these acid metabolites with a deficiency of cocarboxylase following hemorrhage. It is possible that the change in utilization of glucose suggested by Russel, Long, and Engel may be correlated with the increase in the concentrations of lactate and pyruvate found by Govier. The purpose of the present investigation is to determine, by means of simultaneous arterial and venous samples, whether or not hemorrhage is followed by changes in the magnitude of the peripheral glucose utilization and the lactate production of dogs. The effect of hemorrhage upon the blood pyruvate level and the lactate/pyruvate (L/P) ratio was also studied.

**METHOD.** Dogs weighing 9 to 20 kilos, which had been fasted 18 to 24 hours previous to the experiment were used. Various degrees of hemorrhagic shock were produced by Walcott's method (1944). According to his terminology, the bleeding volume is considered to be a 100 per cent hemorrhage. Hemorrhages of varying degrees of severity can then be defined by the fraction of the bleeding volume which is returned. Thus, if 25 per cent of the bleeding volume is returned the animal is said to have undergone a 75 per cent hemorrhage.

All the analyses were done on whole blood. The Somogyi modification (1926) of the Shaffer-Hartman method was used for the determination of blood sugar. The non-fermentable fraction was determined in the blood of 10 dogs before and at hourly intervals after hemorrhage until death occurred. Hemorrhage had no effect on the average control value of the non-fermentable fraction, which has a reducing value equivalent to 27 mgm. per cent of glucose. It is interesting to note that this average value is the same as that found under most conditions in man (Somogyi, 1927). Since the non-fermentable fraction was not altered by hemorrhage, 27 mgm. per cent were subtracted from all the glucose figures.

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<sup>2</sup> Submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, in the Faculty of Pure Science, Columbia University.

Lactates were determined by the Friedman-Graesser method (1933). Pyruvates were estimated by means of a wedge photometer, using Bueding's (1940) modification of Lu's method (1939). All blood samples were drawn in syringes (Friedman and Hangen, 1942) moistened with "Liquaemin." The samples which were to be analyzed for lactate and glucose were taken in 1 cc. calibrated tuberculin syringes, and the proteins were immediately precipitated with sodium tungstate. The samples upon which pyruvate was determined were collected in 2 cc. calibrated syringes and the proteins were precipitated at once with trichloroacetic acid.

The relative reduction in the amount of blood flowing through the femoral vein after a 75 per cent hemorrhage was measured in 4 dogs in which the blood was heparinized in dosages as recommended by Solandt and Best (1940). A cannula was inserted into a superficial branch of the femoral vein until the tip

TABLE 1

*Blood lactate and pyruvate concentrations before and after slight (50%), moderate (63%) and severe (75%) hemorrhage*

	CONTROL			1 HOUR AFTER HEMORRHAGE			4 HOURS AFTER HEMORRHAGE		
	Lac- tate	Pyr- u- vate	L/P	Lac- tate	Pyr- u- vate	L/P	Lactate	Pyr- u- vate	L/P
	mgm. %	mgm. %		mgm. %	mgm. %		mgm. %	mgm. %	
50% hemorrhage, average 5 dogs.....	11.7	1.7*	6.9	18.2			15.3	2.4*	6.3
63% hemorrhage, average 5 dogs.....	10.5	1.5	6.9	35.1	5.3	6.6	42.2	4.0	10.5
75% hemorrhage, lactates on 26 dogs, pyruvates on 20 dogs.....	13.1	1.9	8.4	65.8	4.7†	14.0†	116.6‡	4.7‡	23.6‡

\* These values were obtained on 2 dogs.

† These data obtained on a series of 5 dogs.

‡ These were terminal samples.

reached the main vein. The femoral vein could be clamped centrally and the blood from it collected for a measured period of time. It is recognized that this procedure measures only the relative variations in blood flow.

RESULTS. The control blood lactate usually varied from 4 to 20 mgm. per cent. In one instance it was as high as 40 mgm. per cent. The blood pyruvates in the control determinations ranged between 0.8 and 2.4 mgm. per cent. Control values of 3 to 10 were found for the lactate/pyruvate (L/P) ratio.

The effects of a 50 per cent hemorrhage (returning 50 per cent of the bleeding volume), a 63 per cent hemorrhage (returning 37 per cent of the bleeding volume), and a 75 per cent hemorrhage (returning 25 per cent of the bleeding volume) on the blood concentrations of lactate and pyruvate and the L/P ratio, are shown in table 1. If 50 or 37 per cent of the bleeding volume was returned, the hemorrhage was not fatal. However, when only 25 per cent was returned death resulted. In a series of 5 dogs each of which had 50 per cent of the bleeding volume

\* Liquaemin-heparin in saline, Roche-Organon Inc., Nutley, N. J.

returned, the average blood lactate concentration and the average L/P ratio were within the range of the control values. One hour after bleeding the highest value found for the lactate in one instance was 35.2 mgm. per cent. The average value for lactates on the next day was 10.3 mgm. per cent.

When 5 dogs were subjected to a 63 per cent hemorrhage (bled out and 37 per cent returned), the average blood lactate 4 hours after bleeding had increased from a control of 10.5 to 42.2 mgm. per cent. In one instance the blood lactate had risen as high as 64.0 mgm. per cent (L/P 12.5). In the four hour period after hemorrhage the average L/P ratio had increased from a control of 6.9 to 10.5, a value which is near the upper limit found in control dogs. None of these animals showed evidences of being in a critical state at the end of the 4 hour experimental period. By the next day the blood lactate of 4 dogs had returned to an average value of 11.5 mgm. per cent. During the night one animal died unexpectedly in his cage. The control blood volume as measured by the blue dye T-1824 showed that previous to bleeding this animal had been in a state of dehydration. One hour after hemorrhage the blood volume reduction in the animal that died was 36.4 per cent. During the succeeding 4 hours the volume of blood samples removed was sufficient to increase the calculated reduction to 42.5 per cent. The critical level of 40.0 per cent blood volume reduction (Walcott, 1944) had thus been exceeded during the period of experimentation.

All 26 of the dogs which underwent a 75 per cent hemorrhage died, the average survival time being 3 hours and 20 minutes. During the first hour following hemorrhage, the average blood lactate in these dogs increased from a control value of 13.1 mgm. per cent to between 30.0 and 120.0 mgm. per cent. Subsequently, the lactate continued to rise slowly, a rapid increase occurring terminally, at which time the lactate varied between 70 and 160 mgm. per cent. The blood pyruvates (series of 20 dogs) which had an average control value of 1.9 mgm. per cent increased to 4.7 mgm. per cent one hour after hemorrhage and showed little change thereafter. Since there was little change in the concentration of pyruvate following the initial rise, the L/P ratios increased, and terminally varied between 16.4 and 31.0. Dog 5A (fig. 1) illustrates the typical rapid increase in lactate followed by a plateau which in turn is succeeded by a sharp rise. Two and one-half hours after hemorrhage the L/P ratio was 14.2. At this time the animal was probably suffering from moderate hypoxia, with a blood lactate of 66.6 mgm. per cent and a pyruvate of 4.7 mgm. per cent. Four and one-half hours after hemorrhage the blood lactate was 151.3 mgm. per cent and the L/P ratio 23.6. Shortly thereafter the dog died.

Figure 1 shows that the lactate increased more in the animals which had been subjected to 63 per cent hemorrhages than in those that had lost only 50 per cent of their bleeding volume. Figure 1 also indicates that, in general, the lactate concentrations which are present after a 75 per cent hemorrhage and the rate at which these values are attained are inversely related to the survival time of the animal.

The effect of hemorrhage upon the concentration of blood glucose is highly variable, being dependent on the previous history of the animal, the amount of

liver glycogen available, etc. However, in normal well-fed dogs subjected to a 75 per cent hemorrhage (bled out and 25 per cent returned) the arterial blood sugar rose sharply from control values ranging between 57.0 and 100.0 mgm. per cent to a maximum of between 118.0 and 335.0 mgm. per cent. The maximum occurred at any time following hemorrhage except terminally. There was almost invariably a drop in glucose of 10 to 90 mgm. per cent within the last hour of life. In some instances the terminal decrease took place within the last 10 minutes. Death generally occurred with the blood sugar value slightly greater than that of the control blood samples. The curve for blood glucose in figure 2 shows the typical increase and terminal decrease after a 75 per cent hemorrhage.

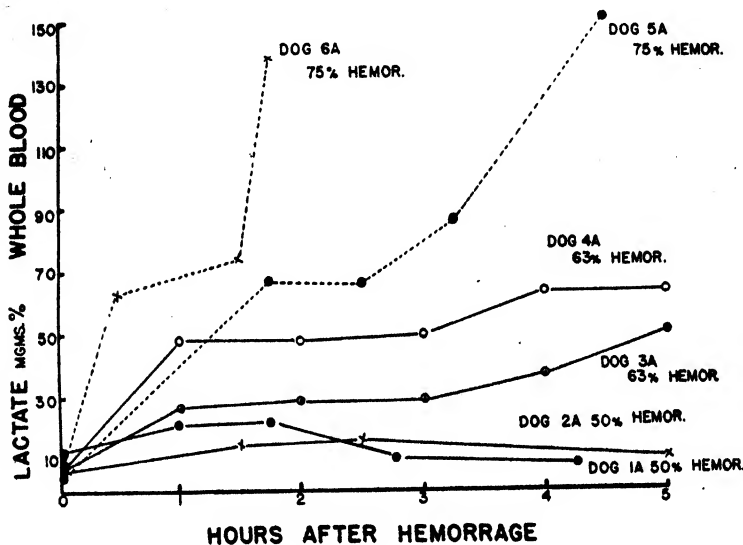


Fig. 1. Showing changes in blood lactate concentrations after slight, moderate, and severe hemorrhage. The dogs which died are indicated by broken lines, those which survived by unbroken lines.

Another series of experiments was performed in order to determine the course of peripheral glucose utilization and lactate production following a 75 per cent hemorrhage. The decrease in blood glucose (A-V difference) as the blood passed through the tissues of the hind limb was arbitrarily termed glucose utilization, although it is recognized that a disappearance of glucose does not necessarily signify utilization. To determine the A-V differences in glucose, simultaneous (within 1 to 3 min.) samples of arterial and venous femoral blood were taken before and after 75 per cent hemorrhages (table 2). Control experiments were carried out in which 2 dogs were placed on the animal board for a period of time corresponding to the duration of an average hemorrhage experiment, and samples were drawn from the femoral artery and vein at hourly intervals. Under these conditions the glucose A-V differences remained relatively unchanged (table 2). Additional control values for the A-V glucose differences were obtained upon

each dog before it was bled. The average decrease of all the control values in glucose from arterial to venous blood was 3.4 mgm. per cent (20 determinations). One hour after hemorrhage the A-V difference had risen to an average of 32.0 mgm. per cent. The average maximum A-V difference of glucose was 40.0 mgm. per cent; the average terminal A-V difference was 19.0 mgm. per cent. The range of the terminal A-V differences was considerable. For example, in dog 11 the glucose A-V difference  $\frac{1}{2}$  hour before death was 7.0 mgm. per cent. In dog 8 it was 34.0 mgm. per cent  $\frac{1}{2}$  hour before death. The maximum A-V difference which occurred at various times after hemorrhage was always followed by a steadily decreasing A-V difference until death occurred (fig. 2). When time

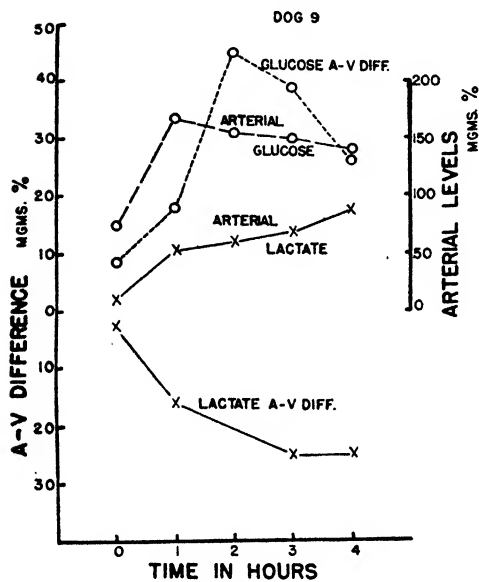


Fig. 2

Fig. 2. Showing changes in the arterial levels of glucose and lactate and the arterial-venous differences in glucose and lactate after a 75 per cent hemorrhage.

Fig. 3. Showing the effect of 75 per cent hemorrhage on the arterial-venous differences in red cell volume. Broken lines indicate the venous hematocrits, unbroken lines the arterial hematocrits.

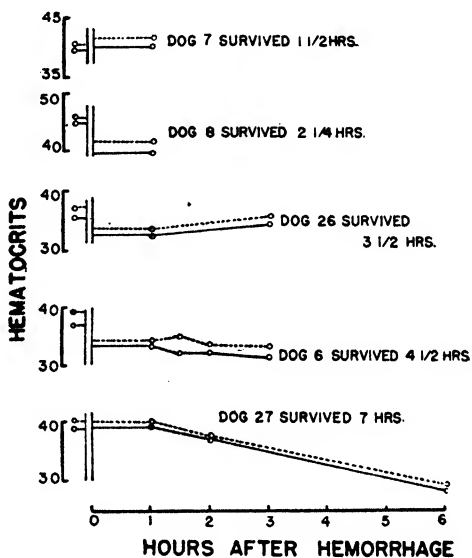


Fig. 3

permitted, terminal samples were taken as the blood pressure fell to between 20 and 15 mm. Hg. If, however, death took place suddenly, samples obtained within  $\frac{1}{2}$  hour of death were considered terminal. The A-V difference bore no constant relation to the absolute level of arterial glucose.

The pattern of glucose utilization following a 75 per cent hemorrhage was unchanged when the duration of fasting previous to bleeding was greater than 18 to 24 hours. For example, dog 14, given water ad libitum but no food for 5 days, had a control glucose A-V difference of 3.0 mgm. per cent and an arterial glucose of 88.0 mgm. per cent (table 2). One hour after hemorrhage the A-V difference had risen to a maximum of 50.0 mgm. per cent. Two hours after



hemorrhage the difference was 23.0 mgm. per cent and one hour later it was 15.0 mgm. per cent. The dog survived  $3\frac{1}{2}$  hours. The results indicate that 5 days of fasting did not change the glucose response to hemorrhage.

The A-V lactate differences were unchanged when a dog remained on the animal board for a period of time corresponding to the duration of the usual hemorrhage experiment. The average A-V difference for these control values as well as those obtained immediately before hemorrhage was 3.0 mgm. per cent (table 3). Since the femoral venous lactate concentration was higher than that

TABLE 2

*The relation of arterial glucose (in mgm. per cent) to the femoral A-V glucose difference after a 75 per cent hemorrhage*

DOG NO.	CONTROL		1 HOUR		2 HOURS		3 HOURS		4 HOURS	
	Arterial	A-V	Arterial	A-V	Arterial	A-V	Arterial	A-V	Arterial	A-V
Control experiments (time on animal board)										
1	81.7	2.4	85.2	2.7	81.7	0.8	80.7	1.6	81.7	0.8
2	72.0	4.0	75.5	7.7	77.7	3.0				
Hemorrhage experiments (time after hemorrhage)										
3	78.5	7.0	118.0	35.0	dead					
4	70.0	3.3	118.0	13.0	dead					
5	72.0	2.0	155.0	12.0	163.0	30.0	180.0	9.0	dead	
6	83.0	4.3	166.0	37.0	150.0	29.0	125.0	23.0	dead	4½hr.
7	91.5	4.3			dead					
8*	75.0	3.0	190.0	54.0	168.0	34.0	dead			
9	73.5	8.5	168.0	18.0	155.0	45.0	148.0	39.0	141.0	26.0
10	78.8	0.0	335.0	27.0	293.0	39.0	265.0	28.0	dead	
11	70.0	4.0	186.0	38.0	125.0	7.0	dead			
12	61.3	0.0	158.0	38.0	69.0	6.0			61.3†	10.8†
13	66.7	3.7	228.0	27.0	168.0	10.0	dead			
Starved for 5 days before hemorrhage										
14	88.0	3.0	193.0	50.0	178.0	23.0	159.0	15.0	dead	

\* 70 per cent hemorrhage.

† This was a 5 hour sample.

present in the femoral artery, the blood has gained lactate in passing through the tissues and the A-V lactate difference may be regarded as negative, when compared with the glucose A-V difference. One hour after a 75 per cent hemorrhage the average A-V lactate difference was 24.2 mgm. per cent; gradually increasing to an average maximum value of 36.5 mgm. per cent. The average terminal A-V lactate difference was 29.5 mgm. per cent. The maximum A-V difference occurred at various times after hemorrhage, sometimes even terminally.

The question arises as to the relation of these changes in A-V differences in glucose and lactate to the changes in the relative blood flow through the femoral

vein. In each of the 4 dogs studied the blood flow through the femoral vein was reduced immediately after a 75 per cent hemorrhage to between 25.0 and 31.2 per cent of the control value. There was no marked change in blood flow following the initial reduction until the blood pressure fell to 15 mm. Hg or below, at which time the blood flow decreased sharply (table 4).

In 5 animals the hematocrit values were determined upon each of the arterial and venous samples (fig. 3). A small difference in the hematocrit reading was observed between the arterial and venous blood, but the difference was unchanged by hemorrhage. These observations indicate that the alterations in

TABLE 3

*The relation of arterial lactate (in mgm. per cent) to the femoral A-V lactate difference after a 75 per cent hemorrhage*

DOG. NO.	CONTROL		1 HOURS		2 HOURS		3 HOURS		4 HOURS	
	Arterial	A-V	Arterial	A-V	Arterial	A-V	Arterial	A-V	Arterial	A-V
Control experiment (time on animal board)										
15	10.5	2.5	9.2	2.2	8.8	2.4	8.2			
Hemorrhage experiments (time after hemorrhage)										
16	12.4	7.3	43.8	18.0	46.8	24.4	47.4	29.1	dead 5½ hr.	
17	16.3	0.7	82.3	45.2	143.9	37.6	dead			
18	33.1	2.4	71.0	33.4	96.4	35.6	dead			
19	11.3	2.9	58.9	21.0	49.7	25.3	66.6	27.0	dead	
3	10.2	1.6	80.6	26.4	dead					
4			73.3	26.3	dead					
5	3.0	4.5	51.6	13.0	66.7	34.5	69.7	25.9	dead	
6	5.2	4.6	42.8	32.0			55.0	30.2	dead 4½ hr.	
7	12.9	3.2	120.0	14.0	dead					
8†	40.5	0.7	108.0	12.0	140.0	62.0	dead			
9	10.4	2.4	54.2	16.3			67.2	35.1	85.3	35.2
10					65.2	26.3	70.5*	42.3*	dead 3 hr.	
11	4.6	4.4	52.9	27.7	114.0	16.0	136.0	7.8	dead	
20	4.0	3.0								
21†	4.1	3.3	55.8	30.8	95.5	14.5	dead 3½ hr.			

\* This was a 2½ hour sample.

† 70 per cent hemorrhage.

blood glucose and lactate A-V differences need not be corrected for changes in red cell volume.

DISCUSSION. After a 50 per cent hemorrhage the blood lactate, the blood pyruvate and the L/P ratio remained within the control ranges. When a greater amount of blood was withdrawn (63 per cent of the bleeding volume), there was a sharp rise in pyruvate, and a moderate increase (4 fold) in lactate, whereas the L/P ratio remained at the upper limit of the control range. A marked increase in blood lactate occurred immediately after a 75 per cent hemorrhage. This was succeeded by a period during which the lactate showed no

increase or rose slowly. Terminally, there was usually a rapid increase in the blood lactate which sometimes attained values as high as 160 mgm. per cent. The average pyruvate concentration was only 0.7 mgm. per cent higher terminally in the dogs which had lost 75 per cent of their bleeding volume than it was after an equal time interval in the dogs that had lost 63 per cent of their bleeding volume. There was a considerable increase, therefore, in the L/P ratio in the dogs with 75 per cent of their bleeding volume removed (table 1). It is apparent that when smaller fractions of the bleeding volume were returned to the dogs, larger changes in blood lactate, pyruvate and the L/P ratio were observed.

Greig and Govier (1943) found a deficiency in cocarboxylase in the muscular tissue of hemorrhaged dogs (nembutal anesthesia). The enzyme deficiency

TABLE 4

*Blood in cc./minute obtained from the femoral vein before and after 75% hemorrhage; duplicate determinations included in the table*

DOG NO.	WT.	CONTROL FLOW	FLOW FOLLOWING 75 PER CENT HEMORRHAGE			
			One hour	Two hours	Three hours	Blood pressure 15 mm. Hg or below
	kgm.	cc./minute	cc./minute	cc./minute	cc./minute	cc./minute
22	7.2	16.0	5.0	4.1*		3.2
		18.0	4.4	4.4		4.0
			4.6			
23	7.5	18.0	5.0	4.5	5.4	2.2
		16.0	5.5	3.6	4.6	1.3†
24	9.5	40.0	10.8	12.4	6.0	7.7
		38.0		12.0	6.8	
25	10.7	42.0				
		21.0	5.5	5.7		5.0
		23.0	5.5			

\* This sample was taken at 1½ hours.

† This sample was taken as the blood pressure dropped from 10 to 2 mm. Hg.

could explain the increase in lactate and pyruvate that occurred previous to the terminal rise in the L/P ratio. Even without an actual decrease or dephosphorylation of cocarboxylase a rise in peripheral utilization of glucose could increase the demand for this enzyme, and thus cause a relative deficiency. It is recognized that cocarboxylase is probably not the only substance concerned with the breakdown of pyruvate (Pilgrim, Axelrod and Elvehjem, 1942). The severe hypoxia that developed terminally is reflected by the marked rise in the L/P ratio.

The maximum A-V difference in glucose following a 75 per cent hemorrhage was approximately 12 times that of the control, an increase which cannot be accounted for on the basis of the observed decrease in blood flow, which amounted to ¼ of the control value (table 4). This decrease in blood flow may be produced partly by an increase in the circulation time, and partly by a decrease in the

total cross sectional area of the vascular bed of the hind limb. The rise in A-V differences indicates an increased peripheral utilization of glucose resulting from hemorrhage, a phenomenon also found in rats by Russel, Long and Engel (1944). Of course, A-V differences measure absorption and not necessarily utilization. That the glucose was stored seems impossible since Tachi (1926) found a marked decrease in the muscle glycogen of rabbits following severe hemorrhage. In dogs that have undergone a 75 per cent hemorrhage the glucose A-V difference rose to a maximum after which there was a steady decrease. Since during this period the blood flow remained constant at about 25 per cent of the control flow, the decrease in glucose A-V difference must represent a definite decrease in tissue metabolism. This decrease in glucose A-V difference does not necessarily mean that the utilization has dropped below the control values, for the average terminal A-V glucose difference was still 5.5 times higher than the control value. The increase in the average terminal glucose A-V difference cannot be accounted for entirely on a basis of decreased blood flow. Nevertheless in individual dogs the A-V values may indicate a terminal decrease or a terminal increase in peripheral utilization as compared with control values. For example, dog 11,  $\frac{1}{2}$  hour before death showed a rise of less than 2 fold in the glucose A-V difference. Less glucose was being utilized by the peripheral tissues in this period than before hemorrhage. On the other hand,  $\frac{1}{2}$  hour before death the glucose A-V difference in dog 8 was 10 times the control difference. At this time the animal was utilizing a large amount of glucose.

Although all the data have not been collected on the same dogs, it is possible to relate the maximum period of glucose utilization following a 75 per cent hemorrhage with the period during which the blood lactate and pyruvate concentrations are rising and the L/P ratio is still less than 14 (table 3). As the hypoxia progresses the L/P ratio rises and the peripheral glucose utilization falls.

According to the A-V differences, lactate was produced, after a 75 per cent hemorrhage, at a rate 12 times greater than in the control period. In some dogs the rate of lactate formation decreased terminally. The fact that the maximum A-V lactate differences did not necessarily occur simultaneously with the maximum A-V glucose differences indicates that there is a changing relationship between glucose utilization and lactate production as measured by blood concentrations.

#### SUMMARY

Dogs were bled out and 25, 37, and 50 per cent of the bleeding volume returned to the circulation. Animals in which less blood is returned show greater increases in the blood lactate, pyruvate and lactate/pyruvate ratio.

After severe hemorrhage (25 per cent of the bleeding volume returned) certain changes in the carbohydrate blood chemistry are found.

1. There is an initial increase in the blood concentrations of lactate and pyruvate with little change in the lactate/pyruvate ratio above the control variation of 3 to 10.

2. A marked terminal increase in blood lactate, with no corresponding terminal increase in pyruvate, raises the lactate/pyruvate ratio as high as 31.

3. A rise in the blood concentrations of arterial glucose is followed by a terminal decrease.

4. The glucose A-V differences rise to a maximum and this maximum is followed by a progressive decrease until death supervenes. The results (A-V differences) are interpreted in terms of an initial increase in peripheral glucose utilization followed by a decrease. In certain animals the terminal glucose utilization falls below the control value.

The author is indebted to Dr. James B. Allison and to Dr. Magnus I. Gergersen for suggesting this problem, and for their advice and encouragement during the course of the research, and also to Dr. Walter S. Root for his valuable assistance in the preparation of the manuscript.

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# OBSERVATIONS ON SHOCK FOLLOWING BILATERAL VENOUS OCCLUSION OF THE HIND-LIMBS OF THE DOG<sup>1</sup>

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Previous work from this department (1, 2) has shown that venous occlusion, by means of ligation and lampblack injections, of a hind-limb in the dog leads to a shock-like state in which 89 per cent of the animals succumb in  $3\frac{1}{2}$  to 21 hours (3). This is associated with a leakage of intravascular fluid into the leg of more than 4 per cent of the body weight. This leakage is attributed to the elevation of the hydrostatic pressure in the small peripheral vessels following occlusion. Later, alterations in capillary permeability in this leg permit proteins and, still later, formed elements to escape. This view is supported by the efficacy of *a*, fluid replacement which permitted 12 out of 12 dogs to survive when intravenous isotonic saline was employed shortly after operation and 5 out of 12 dogs when intravenous isotonic glucose was used (3), and *b*, prevention of fluid escape which permitted survival of 11 out of 12 dogs when rigid plaster casts were applied (4).

In another study (5) we decided to use venous occlusion of both hind-limbs to ensure an early consistently fatal outcome. This suggested that it might be worth while to examine the effects of fluid replacement and of casting on animals in which the traumatic insult was greater, viz., venous occlusion of both hind-limbs. It soon became apparent that the results were very different from those obtained with unilateral venous occlusion. These differences and their possible interpretation are the subject of this report.

**METHODS.** The procedures of inducing the shock-like state have been previously reported (1 to 5). The method of administering saline was as previously described (3) except that larger quantities were given, and the method of applying the cast has been described (4). In one series of untreated animals with bilateral vein occlusions, anesthesia was maintained with intravenous nembutal (25 mgm./kilo.) throughout the duration of the experiment. All other dogs were operated upon under ether anesthesia and ether was discontinued following completion of the surgical procedure. The plasma used in the plasma treated dogs was obtained by bleeding of normal animals, and subsequent centrifugation of the blood followed by separation of the plasma by means of a siphon.<sup>2</sup>

**DISCUSSION OF RESULTS.** The pertinent data on the survival of the various groups of animals are summarized in table 1.

1. *Variation in survival rate of unilateral venous occlusion series.* It is apparent

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from examination of table 1 that the same procedure carried out on different series of dogs did not give identical results. Thus, in our previously reported study venous occlusion of one hind-limb was fatal in 17 out of 19 dogs. When this procedure was repeated in the present series the fatalities were less frequent, 22 out of 28. This difference is slight and can be attributed to the vagaries of random sampling. A greater, but probably insignificant difference, is noted in the saline treated unilateral venous occlusion experiments (table 1). Thus, in the previously reported saline treated animals, all 12 dogs survived. In the

TABLE 1

*Comparison of survival rate in unilateral and bilateral venous occlusion of the hind-limbs in the dog*

TYPE OF VENOUS OCCLUSION OF THE HIND-LIMBS	ADDITIONAL PROCEDURE	ANESTHESIA	NO. OF ANIMALS	NO. OF SURVIVALS	%	REMARKS
Unilateral	None	Ether during operation only	19	2	11	Previously reported (3) Present series
			28	6	21	
			—	—	—	
Unilateral	Isotonic saline	Ether during operation only	47	8	17	Previously reported (3) Present series
			12	12	100	
			28	15	54	
Unilateral	Cast	Ether during operation only	—	—	—	Previously reported (4)
			40	27	68	
			12	11	92	
Bilateral	None	Nembutal throughout	13	0	0	
Bilateral	None	Ether during operation only	6	0	0	
Bilateral	Cast	Ether during operation only	14	1	7	
Bilateral	Isotonic saline	Ether during operation only	8	0	0	
Bilateral	Plasma	Ether during operation only	6	0	0	

present series fatalities within the first 21 hours occurred in 13 out of 28 dogs. The difference in the two sets of experiments, however, does not invalidate the previous conclusion that isotonic saline when administered in repeated doses early after unilateral venous occlusion of a hind-limb has a definite beneficial effect.

2. *Effect of bilateral venous occlusion of the hind-limbs.* No survivals occurred following the venous occlusion of both hind-limbs (table 1). The survival time in the bilateral vein occlusion dogs was shorter than in the single limb vein

occlusion. Thus in the 6 animals which were anesthetized with ether during the operation, the survival time was  $2\frac{1}{2}$  to  $5\frac{1}{2}$  hours, a distinctly shorter interval than in the case of those animals with vein occlusion of a single hind-limb, in which the survival time, in those that succumbed, ranged from  $3\frac{1}{2}$  to 21 hours. A similar result was obtained in the double venous occlusion series in which the animals were maintained anesthetized with nembutal. All 13 dogs succumbed within  $1\frac{3}{4}$  to 8 hours after operation (table 1).

The more accelerated and invariably fatal courses in animals with bilateral vein occlusion is understandable and almost predictable. With the veins of two limbs occluded the loss of fluid into both legs is more rapid and more difficult to compensate for, than in the case of involvement of one limb. Compensatory mechanisms to counteract the fluid loss would furthermore be less apt to be effective.

However, another factor must be taken into account, viz., the blood trapped in the blood vessels of the limb immediately upon venous occlusion. In essence this operates like hemorrhage except that here the "bleeding" is into a part of the circulatory tree whose egress into the general circulation has been cut off, at least temporarily. While the amount of blood trapped in one limb may not be of sufficient magnitude to contribute significantly to the downhill course of the animal, the amount so trapped in both hind-limbs may be large enough to make a noticeable difference and per se should accelerate the development of the shock-like state and so further strain any compensatory mechanisms which would operate.

3. *Effect of early isotonic saline or plasma infusions on the course of the shock and survival of dogs with bilateral venous occlusion of the hind-limbs.* As shown in table 1, isotonic saline administered to animals with bilateral venous occlusion in the same manner as in single limb venous occlusion experiments failed to prevent death in any of the 8 animals and only in one was the duration of survival lengthened outside the range of the untreated dogs, viz., prolonged to 18 hours. This occurred in spite of the fact that the quantity of saline injected was substantially larger than in the single hind-limb vein occlusion experiments. Plasma also failed to prevent a fatal outcome in the six animals in which it was used in a manner and in quantities similar to the saline. All 6 of the plasma treated animals succumbed within the same time period as the untreated animals.

Thus, fluid replacement in the bilateral venous occlusion experiments, whether in the form of isotonic saline or plasma was demonstrated to be without apparent effect on the survival of the animals or on the development of the shock condition. This holds even though the quantity and frequency of fluid administration were greater than in the single hind-limb vein occlusion. This difference between bilateral and unilateral venous occlusion suggests that it is important not only to know during which stage of shock therapy is being administered (viz., "pre-shock", early shock, late shock or the "irreversible stage") but also to know the magnitude of the insult or trigger mechanism leading to shock and its progression. The insult may be slight or severe and accordingly its effect may be slight, moderate or overwhelming. Consequently, therapy



effective in milder cases may fail in more serious ones even if begun early enough. This merits emphasis because it has often been overlooked in the recent tendency to define the stage of progress of the shock condition. The rate of development of shock and the rate of its progression are not the sole criteria of the seriousness of the condition, in terms of reversibility by suitable therapy. Some cognizance must be taken of the magnitude of the insult leading to shock. In the case of venous occlusion of both hind-limbs, the shock appears to be irreversible from the start.

4. *Effect of casting the hind-limbs and abdomen on the course of the shock and survival of dogs with bilateral venous occlusion of the hind-limbs.* As shown in table 1 casting the limbs and abdomen after bilateral venous occlusion had little effect on survival of the dogs, 13 out of 14 animals succumbing. This is in sharp contrast with the effect of similar casting in dogs with unilateral venous occlusion, previously reported (4), in which only 1 out of 12 animals succumbed. Casting, however, did prolong the survival time of these double vein occlusions; the 13 dogs that succumbed, survived for from 5 to about 17 hours (most of them for the longer period) compared to the untreated animals which survived for from  $2\frac{1}{2}$  to  $5\frac{1}{2}$  hours.

The reason for the difference in survival between the two groups of casted dogs cannot be attributed to differences in local fluid loss, since the casts prevented any significant fluid leakage. The difference in survival of the two groups must be attributed to other factors. It is possible that this is the longer period of ether anesthesia, necessary with the double operation. We are inclined to discount this since we have had prolonged anesthesia in a number of single limb occlusion dogs which survived. Another possibility is the one, already mentioned, that the amount of blood taken out of circulation by the double occlusion is increased and may be so great that without the additional insult of fluid leakage it is sufficient per se to lead to shock and death. Casting would not prevent this.

While the foregoing two possibilities cannot be excluded, it is also possible that neurogenic influences may be operating to lead to the progressing shock-state. More likely is the possibility that vein occlusion, like prolonged application of a tourniquet, leads to the formation of altered metabolites in the tissues of the limb which are absorbed and trickle into the general circulation through the undisturbed lymphatic drainage and that these substances are responsible for the fatal outcome in the animals.

In short, these cast experiments demonstrate that some other factor, possibly a "toxic" substance from the limbs with interfered circulation operates to contribute to the development and progression of shock, in addition to the local fluid loss. When fluid loss is prevented in single limb vein occlusion by casting, this accessory factor is not sufficient by itself to lead to shock, but in experiments employing bilateral venous occlusion it is. Evidently some other factor, possibly a humoral "toxic" substance, plays a rôle even in what appeared at first sight to be a simple local fluid loss-engendered-shock. This unexpected finding is, we believe, significant in demonstrating the complexity of the genesis of shock.

## SUMMARY

Bilateral venous occlusion of the hind-limb of the dog by means of ligation and lampblack injection leads to a consistently fatal and rapid shock-like state. Early fluid administration (NaCl or plasma) demonstrated to be effective in preventing this state in unilateral venous occlusion experiments is without beneficial action in these animals. Likewise, application of a rigid cast to both hind limbs and lower abdomen, in order to minimize fluid leakage into the injured area, is accompanied by slightly increased length of life but does not prevent the ultimate fatal result; this suggests that some other factor, perhaps a humoral "toxic" substance plays a role. The mechanisms and possible interpretation of these results are discussed.

We are indebted to Dr. B. Kondo for his assistance in the conduct of these experiments.

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# THE RATE OF CARBON MONOXIDE UPTAKE BY NORMAL MEN

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Fifty years ago Haldane (5) showed that when a normal man at rest is exposed to air containing 1.3 per cent CO he absorbs into his blood 50 to 60 per cent of the inspired CO during the early stages of the exposure. Were it not for the dead space of the lungs, which is usually about one-third the volume of the tidal air at rest, the proportion absorbed would rise to around 80 per cent, as Haldane pointed out. Later workers (2, 3, 9, 11, 12, 17, 18) have extended these observations, which in Haldane's papers were limited to a single individual, and have furthermore studied the effect of varying grades of activity on the rate of CO uptake. Their results are often irregular and somewhat confusing due in part to marked individual variations between the different subjects used, but in the main we believe, to two sources of experimental uncertainty: 1. Normal human blood contains variable traces of CO (i.e., 1 to 5 per cent COHb) even before exposure to the CO-containing atmosphere. Such traces have been frequently neglected in previous work. 2. The determination of the per cent COHb in the blood has often been made by subjective optical or colorimetric methods, which sometimes give unreliable results in the important range of 0 to 25 per cent COHb, even though they may be quite dependable at higher percentages.

The purpose of this paper was therefore to make a new and comprehensive study, by the accurate and objective micro-gasometric methods now available (19), of the rate of CO uptake by man under a wide range of conditions. Six factors have, in all, been studied by us: 1, per cent CO in inspired air; 2, time of exposure; 3, degree of activity of the subject; 4, per cent O<sub>2</sub> in inspired air; 5, total barometric pressure; 6, variations between different individuals. Factors 1 to 3 have been studied by previous workers, and besides being of theoretical interest in regard to gas exchange in the lung, are of considerable practical importance both from the military and industrial angles, since as little as 10 to 15 per cent COHb in the blood may have marked effects on certain functions (especially visual). Factors 4 and 5 have been but little, if at all, studied previously: on the practical side such additional data may prove of value in problems of aviation and submarine medicine. On the theoretical side, as is shown in an adjoining paper, the study of the effect of varying the per cent O<sub>2</sub> in inspired air may well throw some new light on the detailed mechanism of gas exchange between the lungs and blood, notably in regard to the average time which the red cell spends in traversing the blood capillaries in the pulmonary alveoli.

**METHODS.** The experiments on varying the per cent CO in the inspired air, the time of exposure and the degree of activity were divided into two sets. In

the first set the subjects, two or three at a time, entered a chamber which contained the desired percentages. The chamber contained a bed and a bicycle ergometer. Commonly one subject lay upon the bed, another rode the bicycle, and the third moved about the chamber reading instruments and taking blood samples which he passed out through an air lock. Two grades of work were used on the bicycle, one of 2220 foot pounds per minute, the other double this. The lighter grade of work corresponds roughly to walking on the level, the harder to a slow jog trot. Forty cubic millimeter samples of capillary blood from the finger were analyzed for carbon monoxide by the Scholander-Roughton micro syringe method (19) (accuracy of  $\pm 0.3$  vol. per cent, i.e., 1.5 per cent COHb). The concentration of the gas in the inspired air was determined by means of an M.S.A. carbon monoxide indicator which was turned alternately to the chamber and to a standard mixture in a Tissot gasometer. Both the mixture in the chamber and that in the gasometer were made from pure CO generated from formic and sulfuric acids.

In the second set of experiments with higher concentrations where time was a more critical factor, the subjects breathed through a mouthpiece—first air during a control period and then, at the turning of a valve, from a gasometer containing the desired concentration of CO. In these experiments most of the determinations were made upon venous blood by Horvath and Roughton's gasometric method (10:accuracy 0.05 vol. per cent) or by Roughton's combination of the latter with the micro syringe method (Appendix II, 19:accuracy 0.01 to 0.02 vol. per cent for CO contents  $< 2$  vols. per cent). A few determinations were made on simultaneously drawn capillary blood, as in the earlier set, but the results did not differ significantly.

In a third set of experiments tank O<sub>2</sub> (purity  $> 99$  per cent) was substituted for atmospheric air, and mixtures of CO were prepared therewith in the Tissot gasometers as just described, the procedure in all other respects being identical save that the subject ventilated with O<sub>2</sub> instead of air during the control period.

The experiments at low pressure were conducted in a steel chamber, either breathing air at a total pressure of 410 mm. Hg (simulated altitude = 16,000 ft.) or breathing O<sub>2</sub> at a total pressure of 140 mm. Hg (simulated altitude = 40,000 ft.). Douglas bags, filled with suitable volumes of the gas mixtures from the gasometers, were taken into the chamber for the experiment: the COHb determinations were done outside.

In some of the experiments the proportion of CO absorbed from the inspired air was determined as well as the course of the COHb content of the blood. In certain of these cases the expired air was completely collected and its CO percentage determined either by the M.S.A. instrument or by a modification of the hemoglobin absorption method of Sendroy (20): in others the amount of CO absorbed was calculated from the increase in COHb and the blood volume of the subject, the latter having been determined by the T-1824 method of Gregersen (4). We are much indebted to Drs. M. I. Gregersen and W. S. Root for making the required blood volume determinations.

EXPERIMENTAL RESULTS. I. *Effect of per cent CO, time of exposure and degree*

of activity. Upwards of a hundred observations were made on seven healthy male laboratory workers (ages 20–40 yrs.) in atmospheres containing from 0.01 per cent to 2.0 per cent CO at the four grades of activity described above: rest, light activity, light work and heavy work. Nearly half of the results were obtained in light activity (average ventilation 9.5 liters per minute, average pulse rate 80), this being the most common condition of practical exposure to CO.

The average results for light activity with 0.01 to 0.10 per cent CO in the inspired air are presented in figure 1, and for the whole range of activities and inspired CO percentages (0–2.0) in a composite summarizing chart (fig. 2). In figure 1 the increase in per cent COHb as ordinate is plotted against time in minutes as abscissae, the per cent CO in the inspired air being given by the numbers on the curves. It is assumed that the blank per cent COHb originally

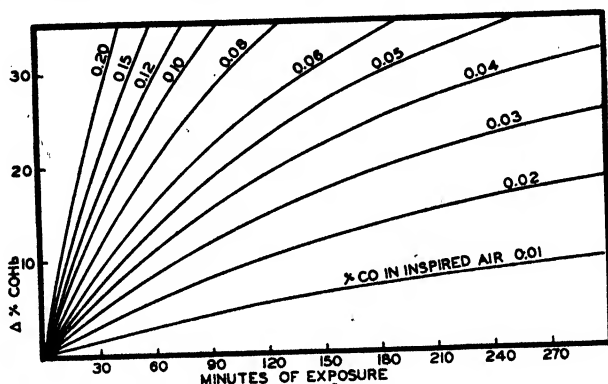


Fig. 1. Uptake of CO by man in light activity

present in the blood is low, i.e., under 10 per cent. The curves in figure 1 are based on the following empirical equation:

(A) Rate of CO uptake =  $k p_{\text{CO}}$

$$\times \frac{\text{per cent COHb at equilibrium} - \text{per cent COHb at time } t}{\text{per cent COHb at equilibrium} - \text{per cent COHb at time zero}}$$

where  $k$  = a constant which varies with the degree activity of the subject, but not with  $p_{\text{CO}}$ .<sup>1</sup>

$p_{\text{CO}}$  = pressure of CO in the inspired air

per cent COHb at equilibrium = the final value which would be reached if the experiment were prolonged until CO uptake ceased.

The last quantity was obtained from the Haldane equation per cent COHb/per cent O<sub>2</sub>Hb =  $M p_{\text{CO}} / \text{arterial } p_{\text{O}_2}$  assuming Sendroy, Liu and Van Slyke's (21)

<sup>1</sup> Actually our data suggest that at the highest concentrations, 1 and 2 per cent CO,  $k$  may increase 10 or 15 per cent, but with high concentrations only short exposures are possible and this introduces technical difficulties which make the observations less certain. This possible rise of  $k$  has been disregarded in plotting the curves.

value of 210 for  $M$  and a value of 98 mm. for the normal arterial  $pO_2$  at sea level (1a, 16). The reasons for choice of equation (A) are discussed later.

Out of a total of forty-one observations during light activity, eighteen were off the curves in figure 1 by 1 per cent COHb or less, nine were off by 2 per cent, nine by 3 per cent, three by 4 per cent, and two by 5 per cent. The average discrepancy from the curves was the same as the average experimental error of the Scholander-Roughton method with 40 cu. mm. blood, namely, 2 per cent COHb. Amongst the higher divergencies are some obvious experimental errors due to the technique being too hurried, but some of the cases are also undoubtedly due to differences between individuals occasioned probably (see Discussion) by varia-

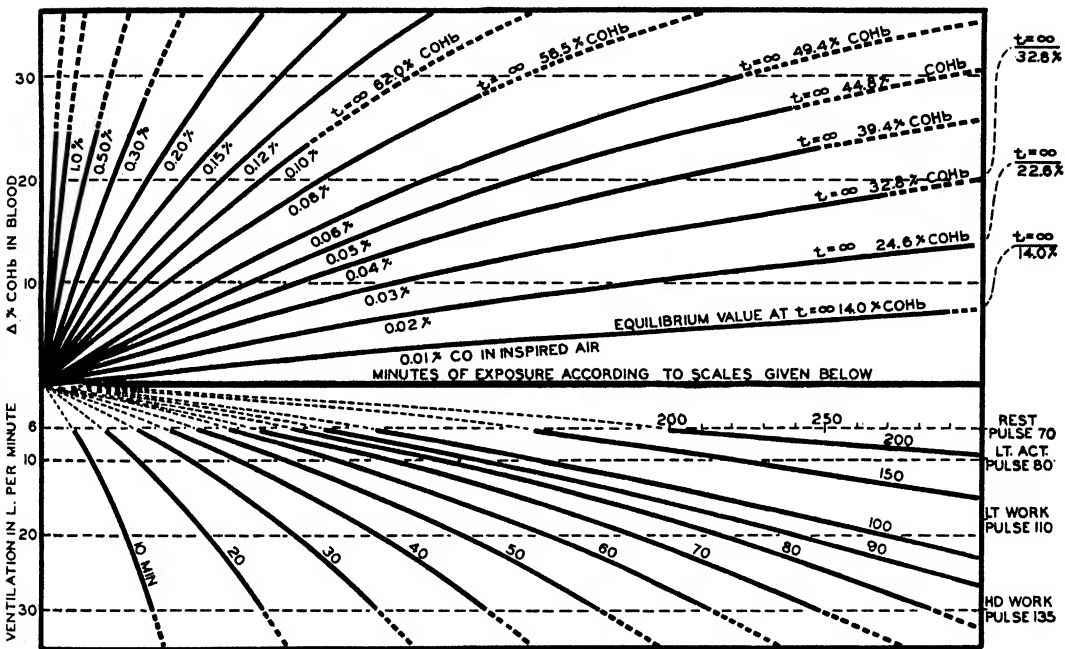


Fig. 2. Uptake of CO by man in various percentages of CO and rates of ventilation

tions in (a) the ratio of the tidal air to the dead space of the lung, (b) the diffusion constant of the lung. In our small group of seven subjects these differences were considerable, e.g., one man rather consistently absorbed 10 to 15 per cent faster than the average and another about the same amount slower. Owing to lack of time and equipment it was not possible to do complete curves on all subjects at all concentrations, and on this account care was necessary to ensure that the data were fair averages in all cases.

Our observations agree with the early results of Haldane in showing that in light activity or rest about 50 per cent of the inspired CO is retained initially, though as equilibrium is approached this ratio naturally drops. A more detailed comparison of our light activity data with those of previous workers at light

activity is given in table 1. It is a representative rather than an exhaustive comparison since we have not included all authors nor all the work of the authors we did consult. Our figures are in fair agreement with Sayers' though slightly higher (17, 18). Henderson's figures (9) are always higher than ours and Killick's (12) for normal subjects are higher still,<sup>2</sup> though Killick's figures for "acclimatized subjects" are lower than Henderson's and are only a little higher than ours for normal subjects. It is interesting that at higher saturations of the blood our data agree more closely with previous work. This is probably due to the two factors mentioned in the introduction, namely, first that the optical or colorimetric methods previously used increase in accuracy as 25 per cent COHb is approached, and second that many previous workers have assumed that the subjects began with no CO in the blood, whereas in the majority of our experiments the initial saturation was measured and found to vary from 0 to 5 per cent. This explains in part the high values found by previous observers in the low ranges.

The whole of our data in all grades of activity is summarized in figure 2 which, by means of two quadrants, gives the relationship of the four variables—CO uptake, per cent CO in the inspired air, time of exposure and ventilation rate or degree of activity. The upper quadrant is exactly similar to figure 1 in the way it relates the increase in per cent COHb to per cent CO in the inspired air and to the time of exposure, while the lower quadrant relates the scale of time to the ventilation. To use this chart one first determines the approximate ventilation and having fixed this point upon the ordinate scale of ventilation one draws a horizontal line to the right intercepting the time curves (marked 10, 20, 30, etc.). The intercepts define the time scale for the ventilation in question and a vertical line, raised from the desired time of exposure on this time scale up to the proper curve in the upper quadrant, will give on the ordinate scale the increase of per cent COHb resulting from this time of exposure. The curved lines in the lower quadrant are time lines, i.e., all points on the 20 minute line are 20 minutes. The solid portions of the curves in both the upper and lower quadrants show the region covered by our experimental data which fit to the curves about as well as was the case with figure 1. The dotted portions are extrapolations. The equilibrium values which each curve approaches and which would be reached at infinite time are indicated after the symbol  $t = \infty$ .

From figure 2 one can thus obtain the increase in per cent COHb when an average normal adult is exposed to any given per cent CO in the air (between 0.01 and 2.0 and at sea level) for any given ventilation rate between 6 and 30 liters per minute. The per cent COHb originally present is assumed to be less than 10. The practical use of figure 2 may be illustrated by the following example:

Find the increase in per cent COHb in the blood of a man, whose ventilation rate is 25 liters per minute, after 85 minutes' exposure to air containing 0.05 per cent CO.

Draw a horizontal line from 25 liters on the lower left hand scale to a point half way

<sup>2</sup> In fact so high that the amount of CO absorbed by the blood in the first half hour exceeded the total amount in the inspired air unless the ventilation was well over 15 liters per minute.

between the 80 and 90 minute lines. Then go up vertically to the 0.05 per cent curve in the upper half of the figure and thence horizontally to the left to the  $\Delta\text{COHb}$  scale. The answer is 28.5 per cent COHb.

The curvature of the time lines in the lower quadrant of figure 2 is due to the fact that with increasing work and ventilation rate the proportion of the inspired CO which is absorbed is found to drop until in the hardest work an average figure of 40 per cent is reached as compared with 50 per cent in rest. The reason for such drop is that diffusion through the lung membrane becomes progressively more important: thus to take an extreme case, with an infinite ventilation rate,

TABLE 1  
*Light activity—per cent COHb in blood*

% CO	STUDY OF	1 HOUR	2 HOURS	3 HOURS	4 HOURS	5 HOURS	6 HOURS
0.02	Present data	6	10.6	14.3	17.2	19.6	21
	Sayers (17)	8	10	18	20		25
	Henderson (9)	11.5					
	Sayers (18)	7.3			13.0		17.6
	Killick (11, 12)	21	25	28	33	33	33
0.03	Present data	9	15	20	25	26	
	Sayers (17)	5.5	11.8	19.8	25.4		
	Henderson (9)	12.3					
	Sayers (18)	10.5	15.8	18.5	23.2	26.5	
0.04	Present data	11.3	20	26.3	31		
	Sayers (17)	8.7	16.2	22.7	27.4		
	Henderson (9)	18.0					
	Sayers (18)	16.3	24.0				
0.06	Present data	16.4					
	Henderson (9)	19.2					
0.08	Present data	21.3					
	Henderson (9)	29.9					
0.10	Present data	26.0					
	Henderson (9)	38					
	Forbes (3)	26.4					

the alveolar  $p\text{CO}$  could not exceed the inspired  $p\text{CO}$ , the volume of CO which could diffuse through the lung membrane must therefore remain finite, so its ratio to the infinite volume of CO inspired would thus tend to zero.

Theoretically the rate of CO uptake should, indeed, be proportional to the difference between the alveolar  $p\text{CO}$  and the average back pressure of CO in the blood in the lung capillaries. Both these quantities increase during the course of an actual experiment: the back pressure at first rises slowly and then with increasing rapidity as the saturation increases because of the nature of the reversible equilibrium between CO and Hb. The alveolar pressure rises because



progressively less CO is abstracted by the blood from the inspired air. It proved extremely complicated to reduce this situation to a quantitative theoretical equation, and instead we have fallen back on the semi-theoretical empirical equation (A), used in figures 1 and 2. In this equation the term  $p\text{CO}$  was inserted to take account of the fact, established by our own and previous work, that the initial rate of CO uptake is proportional to the CO pressure in the inspired air: the term in the bracket represents an effort to allow for the increase in back pressure of CO as equilibrium is approached, for as will be easily seen it decreases from a value of unity at zero time, to a value of zero at infinite time. No provision, however, was made in equation (A) for the rise in alveolar  $p\text{CO}$  during the progress of the experiment: this is one reason, we believe, why the experimental curves usually tend to be more linear in their lower portions than equation (A) indicates. A second factor working in the same direction, especially during short exposures to relatively high CO-containing atmospheres, is the variation in speed of circulation of different parts of the blood: although the circulation time at rest is about one minute, appreciable parts of the blood have a much longer circulation time than this and hence will remove some of the CO picked up in the lungs by the more quickly circulating blood, thus retarding the rise of back pressure of CO in the latter. In prolonged exposure to very low CO-containing atmospheres still another factor enters, namely, the slow disappearance of CO from the blood by channels other than the lungs as demonstrated recently by Roughton and Root. As a matter of fact for per cent COHb increases up to 15 per cent our data can be expressed with an accuracy of 1 in 5 by the following simple formulae, which are thus good enough for many practical purposes, particularly in view of the variability between individuals.

For an individual at rest (ventilation 6 liters per minute, pulse 70) the percentage saturation of the blood (per cent COHb) is given by the concentration of CO in the inspired air in per cent, (per cent CO), times the minutes of exposure ( $t$ ), multiplied by 3, or

$$\text{Per cent COHb} = 3 \times \text{per cent CO} \times t$$

Similarly for light activity (ventilation  $9\frac{1}{2}$  liters per minute, pulse 80)

$$\text{Per cent COHb} = 5 \times \text{per cent CO} \times t$$

For light work (2220 foot pounds per minute, ventilation 18 liters, pulse 110)

$$\text{Per cent COHb} = 8 \times \text{per cent CO} \times t$$

For heavy work (4450 foot pounds per minute, ventilation 30 liters, pulse 135)

$$\text{Per cent COHb} = 11 \times \text{per cent CO} \times t$$

These formulae assume that the initial per cent COHb  $< 5$  per cent and that the per cent CO in the inspired air is not less than 0.02. For 0.01 per cent CO the formulae hold only up to about 7 per cent  $\Delta$  COHb. If the per cent CO is 0.1, the range of applicability is extended up to 30 per cent COHb, and to correspondingly higher values as the per cent CO is further increased.

II. *The effect of O<sub>2</sub> at rest and at hard work.* Roughton (14) has shown that the rate of chemical reaction of CO with hemoglobin in presence of O<sub>2</sub> varies inversely as the O<sub>2</sub> pressure, being equal to  $m' p\text{CO} [\text{O}_2\text{Hb}]/p\text{O}_2$  where  $p\text{CO}$ ,  $p\text{O}_2$  are the respective pressures of CO and O<sub>2</sub>,  $[\text{O}_2\text{Hb}]$  is the concentration of oxyhemoglobin, and  $m'$  is the velocity constant of the reaction. It might therefore be expected that when a subject breathes a given per cent CO in O<sub>2</sub> instead of in air the rate of CO uptake would be reduced, for owing to the slowing of the reaction of CO with the hemoglobin of the red cells when the latter are exposed in the lungs to nearly pure O<sub>2</sub> there would be a correspondingly greater possibility of CO accumulating in physical solution in the blood and developing an appreciable back pressure in the blood capillaries of the lungs. If such an effect were to occur, it should be more marked during work when the rate of CO uptake is some three times greater than at rest. Such was found to be the case in the experiments now to be described: in all of these the period of breathing was so chosen that the blood COHb did not rise above one-third of its equilibrium value, and, in consequence, the rate of COHb increase was found to be approximately linear with the time even in the most unfavorable case—namely, during hard work breathing 98 per cent O<sub>2</sub>.

1. *Experiments at rest.* Several comparisons were made of the rate of CO uptake from mixtures of this gas in air and in 98 per cent O<sub>2</sub>, ranging from 0.1 to 1.2 per cent CO. The initial rate of CO uptake in presence of O<sub>2</sub> was found to be just detectably less than in presence of air (i.e., of the order of 5–10 per cent) in the case of the three subjects studied, W. F., F. C. and F. S. In each case, however, the ventilation rates when breathing O<sub>2</sub> were about 15 per cent higher than when breathing air: the reason for this is not known, though it may have been that the subjects happened to be in a less basal condition in the oxygen than in the air experiments. To allow for the difference reference must be made to figure 2 which shows that with a 15 per cent increase in ventilation rate the rate of CO uptake should, at rest, be increased nearly 15 per cent. On this basis it follows that with equal ventilation rates the CO uptake in presence of O<sub>2</sub> would have been 20 per cent rather than about 5 per cent lower than in presence of air. To clinch this point two specially accurate and well-controlled experiments were done on the subjects W. F. and F. C. The results are given in table 2. In the case of W. F. the ventilation rate was practically the same in O<sub>2</sub> and in air, but the rate of CO uptake was 20 per cent less. In the case of F. C., by way of contrast, the ventilation rate in O<sub>2</sub> was 20 per cent higher than in air whereas the CO uptake rates were almost the same. The final ratios, with correction for the ventilation rates, are 0.76 and 0.78 respectively. These are in close agreement with the previous rougher results and leave no doubt of there being a definite effect of O<sub>2</sub> even when at rest.

2. *Experiments at work.* A similar set of comparisons was done on the subjects W. F., J. E. and F. M. at the standard degree of hard work. The results are given in table 3. In the case of W. F. and J. E. the ventilation rate was practically the same with air as with O<sub>2</sub>, but with F. M. the rate in air was a third greater than in O<sub>2</sub>, so that in this case an appreciable correction is required

on the basis of figure 2 (see last line in table 3). The average ratio for the CO uptake rate in O<sub>2</sub>/CO uptake rate in air is seen to be 0.62. The importance of the back pressure of CO in the blood of the lung capillaries is therefore more pronounced in the case of CO uptake during hard work than during rest. The

TABLE 2  
*Relative rates of CO uptake in air and in oxygen during rest*

Relative rates of CO uptake in air and in oxygen during rest				
	W. F.		F. C.	
% O <sub>2</sub> in inspired air	20.6	98	20.6	98
% CO in inspired air	0.3	0.3	0.3	0.3
Minutes of breathing	10	10	10	10
Δ vols. % CO in blood per minute	0.198	0.159	0.174	0.161
Ventilation rate (liters/minute)	5.86	6.24	5.88	7.05
Blood volume (cc.)	5800		6800	
cc. CO uptake per minute	38.4	30.8	39.4	36.5
% CO in atmospheres				
CO intake rate in O <sub>2</sub>	0.81		0.93	
CO uptake rate in air				
CO uptake rate in O <sub>2</sub>	corrected for difference in			
CO uptake rate in air				
ventilation rates	0.76		0.78	
Average ratio	0.77			

TABLE 3  
*Relative rates of CO uptake in air and in oxygen during hard work*

Relative rates of CO uptake in air and in oxygen during work						
	W. F.		J. E.		F. M.	
	20.8	98.0	20.8	98.0	20.8	98.0
% O <sub>2</sub> in inspired air.....	0.11	0.125	0.127	0.127	0.149	0.146
% CO in inspired air.....	16	6	4.5	4.2	3	12
Minutes of breathing.....	0.27	0.20	0.38	0.22	0.37	0.22
Δ vols. % CO in blood per minute.....	30	32	45	48	64	48
Ventilation rate (liters per minute).....	5800		5100		5700	
Blood volume (cc.).....						
cc. CO uptake per minute.....	142	93	155	89	143	86
% CO in atmospheres.....						
CO uptake rate in O <sub>2</sub> .....	0.65		0.58		0.60	
CO uptake rate in air.....						
CO uptake rate in O <sub>2</sub> .....						
CO uptake rate in air.....	0.63		0.57		0.66	
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theoretical significance of the results of tables 2 and 3 is discussed in an accompanying paper, where they will be made the basis for computation of the average times spent by the red blood cells in the lung capillaries in intimate contact with the alveolar air.

III. *The effect of lowered barometric pressure and simulated altitude.* Three subjects (J. E., F. M. and P. K.) were tested at sea level and in the pressure chamber at a pressure of 410 mm. Hg (simulated altitude = 16,000 ft.) breathing air containing 0.3 to 0.4 per cent CO. The subjects were in a state of light activity and, in the case of the pressure chamber experiments, breathed the ordinary air of the chamber for a few minutes before being connected to the Douglas bags in which the CO air mixtures had been prepared. The period of breathing the CO mixtures was in every case six minutes, and the blood samples collected before and soon after the breathing period showed increases ranging from 1.2 to 2.3 vols. per cent CO. These changes were measured to an accuracy of about 1 in 50 by means of the combined Van Slyke-syringe technique. The results are shown in the columns headed 0 and 16,000 in table 4.

TABLE 4

*Effect of atmospheric pressure and simulated altitude on the rate of CO uptake during light activity*

	J. E.			F. M.		P. K.		W. F.	
	0	16000	40000	0	16000	0	16000	0	40000
Equivalent altitude in feet.....	760	412	140	760	412	760	412	760	140
Barometric pressure.....	20	10.7	12.2	20	10.7	20	10.7	20	12.2
Tracheal O <sub>2</sub> in % atmosphere.....	0.320	0.210	0.091	0.380	0.193	0.38	0.193	0.261	0.091
Tracheal CO in % atmosphere.....	0.234	0.222	0.089	0.320	0.207	0.350	0.215	0.258	0.110
Δ vols. % CO in blood per minute.....	8.2	14.1	11.3	9.8	13.2	9.8	14.2	9.5	10.5
Ventilation rate (ambient liters per minute).....	5100			5700		6100		5800	
Blood volume (cc.).....									
cc. CO uptake per minute	37.7	54.3	50.1	48.2	61.5	56.5	68.3	58	70.8
CO in % atmospheres									
cc. CO uptake per minute									
CO in % atmospheres									
to standard ventilation rate of 10									
ambient liters per minute.....	44	42	42	49	50	57.5	52	61	67
Average ratio.....	42.5			49.5		54.8		64	

For a satisfactory comparison between the results at sea level and at 16,000 feet it is necessary to use the tracheal CO and O<sub>2</sub> pressures rather than the actual percentages in the inspired air. The following formulae were used for the calculations of the tracheal pressures:

Tracheal pCO = per cent CO in dry inspired air

$$\times \frac{\text{Barometric pressure (mm. Hg)} - 47}{760}$$

Tracheal pO<sub>2</sub> = per cent O<sub>2</sub> in dry inspired air

$$\times \frac{\text{Barometric pressure (mm. Hg)} - 47}{760}$$

The factor 47 represents the aqueous vapour pressure at body temperature. It is also necessary to express the ventilation rate per minute in liters measured at the ambient pressure in each case, i.e., 760 mm. at sea level and 412 mm. at the simulated altitude. It will be seen that the ventilation rate at 16,000 feet so measured is distinctly higher than at sea level, thus indicating very appreciable hyperventilation due to the lowered partial pressure of oxygen. The eighth line in table 4 (cc. CO uptake per minute/CO in per cent atmospheres) is calculated by multiplying the  $\Delta$  vols. per cent CO in blood per minute (fifth line) by the blood volume (seventh line) and dividing by the tracheal CO (fourth line). It is appreciably higher at 16,000 feet than at sea level but when the results are corrected to a standard ventilation rate of 10 liters (ambient pressure) per minute, then, as the ninth line of table 4 shows, the difference disappears, the rates at sea level and 16,000 feet agreeing within 0 to 10 per cent, i.e., within experimental error. It may thus be concluded that a lowering of the barometric pressure to 410 mm. Hg and a halving of the partial pressure of  $O_2$  have no significant effect on the rate of CO uptake at a given tracheal  $pCO$ , aside from the changes caused in the ventilation rate by the hypoxia.

Two similar experiments were done on W. F. and J. E. at a barometric pressure of 140 mm. Hg with the subjects breathing  $O_2$  (simulated altitude of 40,000 ft.). The results are given in table 4 under the columns headed 40,000. The ventilation rate is again increased, owing to the lowered partial pressure of  $O_2$  (i.e., 12.2 per cent atmosphere) as compared with sea level, but when this is allowed for the CO uptake rate/tracheal CO in per cent Atmosphere again comes out within 0 to 10 per cent of its value at sea level. There is thus no doubt that diminished barometric pressure per se has an insignificant effect on the rate of CO uptake during light activity.

We have no similar data on the effect, if any, of increased barometric pressure owing to lack of a pressure chamber suitable for such experiments, but the likelihood of any positive effect in such tests would seem extremely remote.

**FURTHER DISCUSSION AND CONCLUSIONS.** The data of this paper show that the initial rate of CO uptake when exposed to atmospheres containing 0.01 to 2.0 per cent CO is proportional to the partial pressure of CO, and increases with the ventilation rate though at a slower rate than the latter. It is independent of the  $O_2$  pressure and the barometric pressure when these are below their normal values at sea level, provided that allowance is made for the increase in ventilation rate if hypoxia occurs. At high pressures of  $O_2$  the CO uptake rate is appreciably reduced, especially in hard work: this decrease, which occurs right from the start, is due to the slowness of the reaction of CO with the hemoglobin of the cells in presence of high partial pressure of  $O_2$ .

Marked individual differences in the rate of CO uptake (i.e., a spread of 10-15 per cent on either side of the mean) have already been recorded and commented on in the first part of this paper during the description of the early rougher results. A similar spread (cp. table 4) was also noticed in the course of the later more accurate work on the subjects referred to in the latter half of the paper. To elucidate the detailed cause of these individual variations more fully

a special pair of experiments was carried out at rest on the slowest absorber of our series of subjects, J. E., and one of our two fastest absorbers, F. C. In addition to the usual determinations, alveolar air samples were collected by the Haldane-Priestley technique at the end of a normal expiration just as the ten-minute period of CO-breathing was being completed. These expiratory alveolar CO percentages have been used to calculate the Dead Space and Diffusion Constant of the lungs and give, we believe, reasonably valid comparative figures for different individuals. To obtain the "absolute" values of the Dead Space and Diffusion Constant the average alveolar CO percentage during the respiratory cycle should be used and this, being higher than the expiratory alveolar CO percentage would lead to lower values of the Dead Space and the Diffusion Constant than those given in table 5. We have not attempted these more refined calculations in view of the doubt as to whether the true average composition of the alveolar air can be accurately determined. The results, based on the ex-

TABLE 5  
*Individual differences in CO uptake between two subjects at rest*

	J. E.	F. C.
% CO inspired air.....	0.259	0.302
% CO expired air.....	0.106	0.102
% CO alveolar air.....	0.035	0.042
Ventilation liters/minutes.....	5.17	5.85
Respirations/minute.....	10.2	10.0
Volume of each breath (cc.).....	506	585
Dead space (cc.).....	160	135
CO uptake per minute (cc.).....	7.75	11.85
Diffusion constant of lungs.....	30	39

piratory alveolar CO percentage are shown in table 5. The dead space of the lungs is calculated from Bohr's formula:

$$\begin{aligned} & \text{Volume of breath} \times \text{per cent CO in expired air} \\ &= (\text{Volume of breath} - \text{dead space}) \times \text{per cent CO in alveolar air} \\ &+ \text{dead space} \times \text{per cent CO in inspired air} \end{aligned}$$

The Diffusion constant of the lungs is calculated from the formula:

$$\text{Diffusion constant} \times \text{per cent CO in alveolar air} \times (\text{barometric pressure in mm. Hg} - 47) = \text{cc. CO taken up per minute}$$

It will be seen from table 5 that although the rates of respiration are the same in the two subjects, the volume of each individual respiration is appreciably less and the dead space greater for J. E. than for F. C. This means that each inspiration brings considerably less of the CO-containing air into the alveoli of J. E. than of F. C., the comparative figures working out at 346 cc. and 450 cc. respectively. This together with the smaller diffusion constant in the case of J. E. explains, we believe, his markedly lower rate of CO uptake. In general,

it seems reasonable to conclude that a slow rate of CO absorption correlates with a low ratio of tidal air to dead space and/or a low diffusion constant.

The numerical values of the dead space given in table 5 are probably too high through the use in Bohr's formula of values for the alveolar per cent CO lower than the average values of this quantity (see discussion above) but they nevertheless fall nicely within the range given by the usual methods on normal individuals. The values of the diffusion constant in table 5 are likewise probably too high and for the same reason, but they also lie within the normal range of 25 to 50 as determined by Krogh (13).

We have, as yet, done no alveolar CO determinations during hard work, but the average value of the alveolar per cent CO can in this case be calculated from Bohr's formula fairly accurately if the dead space is roughly known. Thus in the case of the experiment on J. E. in table 3 the per cent CO in the inspired air = 0.127, the per cent CO in the expired air = 0.077 (by calculation from the per cent CO in the inspired air, the ventilation rate, the blood volume and the rate of increase of blood CO content).

Assuming a dead space of 150 cc. the average alveolar per cent CO by Bohr's formula = 0.072; for a dead space of 200 cc. the alveolar per cent CO comes out to 0.071 and to 0.069 with a dead space of 300 cc. Recent work confirms the view of Krogh, rather than that of Haldane, that the increase in the dead space during work does not exceed 50 per cent of its resting value. On this basis the average alveolar per cent CO in the work experiment on J. E. must have lain between 0.067 and 0.072, and the corresponding value for the diffusion constant comes out between 38 and 42, and is again well within the range of Krogh's values for subjects working at sea level.

Harrop's data (8) on the diffusion constant of acclimatized subjects in the Andes indicate that in rest and light activity there is no significant change in the diffusion constant as compared with the sea level values: the results given in table 4 of this paper point to the same conclusion as regards unacclimatized subjects. No data have, however, to our knowledge been reported as yet on the rate of CO uptake during exercise at low  $O_2$  pressures, and if other work permits we hope to make such tests in the near future. The desirability of such measurements is shown by a recent note of Roughton (15) who shows that diffusion constants of the order of 200 are required if the rate of uptake of  $O_2$  during work at low  $O_2$  pressures is to be explained on the diffusion theory of the passage of gases through the lung membrane. The highest values reported by Krogh during work at sea level are only in the range of 60 to 70, i.e., about one-third of those required according to present calculations if diffusion is to be an adequate mechanism for the uptake of  $O_2$  by man under the most stressful conditions.

Haldane (6) has criticized the Bohr-Krogh method (1, 13) of measuring the diffusion constant of the lungs on the ground that the speed of mixing of gases in the alveoli is not fast enough to ensure a uniform value of the per cent CO throughout the alveoli. This is especially so just after inspiration of a mixture relatively rich in CO. Elsewhere, however, he has pointed out (7) that the diffusion coefficient of a gas is inversely proportional to the total atmospheric pressure,

and therefore that the speed of mixing of air in the lungs would be much improved at low total pressures. He has, in fact, used this consideration to explain a famous experiment of Paul Bert upon the deleterious effect on a bird exposed to total air pressure of 220 mm. Hg of suddenly admitting nitrogen up to a pressure of one atmosphere. Although the kinetics of the mixing of air in the lungs have not yet been finally worked out, it seems clear that if Haldane's contention were correct the rate of CO uptake at a given tracheal pressure of CO should be appreciably greater at a simulated altitude of 40,000 ft. (i.e., 0.18 atmosphere) than at sea level. Table 4, however, shows that when the effect of differing ventilation rates is allowed for, the differences between the CO uptake rates at sea level and at 40,000 feet become insignificant.

#### SUMMARY

1. New data are presented for the rate of carbon monoxide uptake by normal men at sea level, when exposed to air containing various percentages of carbon monoxide (0.01 to 2.0) and for various times. The subjects were at rest, light activity, light work or hard work.

2. A composite chart is given for calculating the average individual increase in percentage COHb in the blood with time, at varying CO pressure and varying ventilation rate. Particular individuals may however vary consistently by as much as  $\pm 20$  per cent from the data in the chart, which may therefore, in practical cases, often be replaced by much simpler approximate equations given in the text. Variations in the ratio of tidal air to dead space, and in the value of the diffusion constant of the lungs appear to be responsible for the differences between individuals in the rate of CO uptake.

3. The observed rates of CO uptake are lower than the average rates of most previous observers: the difference is attributed partly to more accurate estimation of COHb (by the Scholander-Roughton technique) and partly to adequate allowance for the blank CO already present in the blood before the exposure.

4. Lowering of the total barometric pressure (down to 140 mm. Hg) is without effect on the rate of CO uptake, provided the partial pressure of CO in the trachea is kept constant and correction is made for any increase in ventilation rate due to hypoxia.

5. Increasing the O<sub>2</sub> from 20 to 98 per cent at sea level decreases the rate of CO uptake: the effect is more pronounced in hard work than at rest. This decrease occurs because the rate of reaction of CO with Hb is inversely proportional to the O<sub>2</sub> pressure.

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# THE KINETICS OF THE REACTION $\text{CO} + \text{O}_2\text{Hb} \rightleftharpoons \text{O}_2 + \text{COHb}$ IN HUMAN BLOOD AT BODY TEMPERATURE

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Ten years ago I published a series of papers (10) on the rates of the reactions of carbon monoxide with hemoglobin. The reactions studied were *a*, the combination of CO with reduced hemoglobin; *b*, the displacement of  $\text{O}_2$  from combination with hemoglobin by CO; *c*, the displacement of CO from combination with hemoglobin by  $\text{O}_2$ . The technique employed was the early form of the Hartridge-Roughton (4) method of measuring the velocity of very rapid chemical reactions: this method required several liters of solutions for each experiment, and slaughter house blood (usually sheep) was therefore used as the source of hemoglobin. The results were analysed by the usual kinetic methods of physical chemistry and threw new light on the nature and mechanisms of the reactions of hemoglobin with CO and  $\text{O}_2$ . They were not, however, directly applicable to the analysis of the factors, which determine the rate of CO uptake and elimination in man during or after exposure to CO-containing atmospheres, since sheep and not human hemoglobin was studied, and furthermore nearly all the work was done on hemoglobin in solution at  $10^\circ\text{C}$ . to  $20^\circ\text{C}$ . rather than on the red cell at  $37$  to  $38^\circ\text{C}$ .

In the present paper I present some data on the rate of the reaction  $\text{CO} + \text{O}_2\text{Hb} \rightarrow \text{O}_2 + \text{COHb}$  in human hemoglobin solutions and red cells at  $37^\circ\text{C}$ . From a physico-chemical point of view the data are not complete but those already obtained seem sufficiently numerous and definite to justify their use in several interesting physiological problems. This fact, together with the uncertainty as to when the circumstances of the present emergency will permit the completion of the missing physical-chemical aspects of the work, makes it seem desirable to present forthwith a preliminary account of the kinetic results so far obtained. The application of the data to a hitherto unsolved problem, namely, the calculation of the average time spent by the red cell in traversing the capillaries of the lung, is, for the convenience of more physiologically minded readers, presented in an adjoining paper; here also will be found some further considerations upon the factors which determine the rate of CO uptake and elimination in the lung, for the appraisal of these factors requires knowledge not only of the kinetic data of the present paper but also of the average time of the lung capillary circulation.

**EXPERIMENTAL METHODS.** *Measurement of the velocity of the reaction  $\text{CO} + \text{O}_2\text{Hb} \rightarrow \text{O}_2 + \text{COHb}$ .* The technique adopted was a modification of the early form used by Hartridge and Roughton (4). The general layout of the apparatus is shown in figure 1. The two reacting solutions, viz., saline solution equilibrated with an appropriate CO- $\text{O}_2$  mixture, and blood solution or suspension are prepared in separate  $2\frac{1}{2}$  liter glass bottles A and B. These bottles are stoppered

with two-holed rubber bungs, the two leads through which connect on the one hand with a reservoir of compressed air or  $O_2$  and on the other hand with either arm of a simple 2 mm. bore glass T-tap which serves as mixer. The stem of the tap is joined to an observation tube of 5 mm. internal diameter and length about 30 cm. The solutions are driven from their respective containers *A* and *B* into the glass mixer by applying a pressure of 30 to 40 cm. Hg from the reservoir bottle and opening the hemostats on the rubber connections to the mixer. The two solutions emerge from the latter completely mixed and thence travel up the observation tube, wherein readings of the per cent COHb in the running fluid are made at various measured distances from the mixer by means of the Hartridge Reversion Spectroscope for CO determination. Such readings, together with a

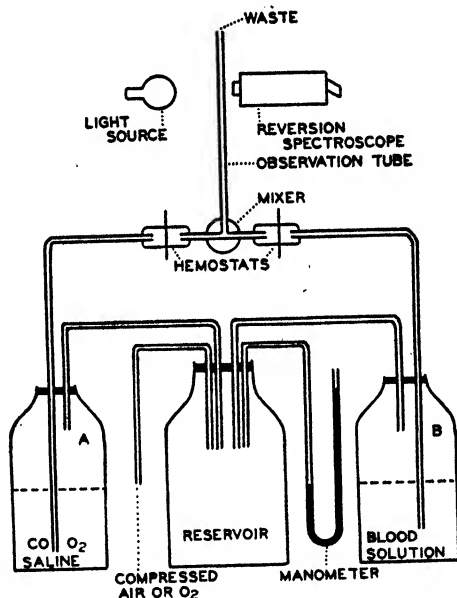


Fig. 1. Apparatus for measuring the velocity of the reaction  $CO + O_2Hb \rightarrow O_2 + COHb$  in solution or in cells.

knowledge of 1, the rate of streaming of the fluid along the observation tube (usually 15 to 20 cc. per second, corresponding to a linear rate of 75 to 100 cm. per second); 2, the relative deliveries of the two reagents from bottles *A* and *B*, and 3, the total concentration of each reagent—viz., CO,  $O_2$  and hemoglobin give all the data necessary for measuring the velocity of the reaction. The experiments of this paper were all done at 37 to 38°C., the whole apparatus being set up, and the observations taken, in a hot room at this temperature. For further information as to the general principles of the method and the calculation of the results reference should be made to our earlier papers (10). Some additional details however, require mention as to the reagents, etc., used in the present work.

1. *Preparation and estimation of the CO-containing solution in bottle A.* Bottle *A* was evacuated, transferred to the hot room and 2000 cc. of warm, Ringer-

Locke solution of usual strength, or of 2 to  $2\frac{1}{2}$  times usual strength run in. The vacuous phase above the solution was then brought to atmospheric pressure by admitting CO gas (prepared from formic and sulfuric acids) or mixtures of CO with  $\text{O}_2$  or  $\text{N}_2$ . The bottle was then rolled on its side for at least five minutes so as to saturate the solution with the gas mixture. The  $\text{O}_2$  and CO contents of the solution after this equilibration were determined by evacuating 5 cc. samples in the Van Slyke apparatus and estimating the  $\text{O}_2$  content in the usual way. The CO content of the gas remaining in the Van Slyke chamber after removal of the  $\text{O}_2$  was obtained by expelling an aliquot portion into the Scholander-Roughton micro-gasometric apparatus and analysing it for CO in the manner described in Appendix II of their paper (11).

2. *Preparation and estimation of the red cell suspension or blood solution in bottle B.* In the case of the red cell suspension experiments 2000 cc. of warm Ringer-Locke solution (usual strength) were equilibrated with air or  $\text{O}_2$  by 5 minutes' rolling in the hot room. Thirty to 50 cc. of freshly drawn human heparinized blood (from an antecubital vein) were then added and mixed with the Ringer-Locke solution by a quick roll. This was repeated periodically during the experiment to prevent settling of the red cells. The usual strength of Ringer-Locke solution was used in bottle A.

For the hemoglobin solution experiments distilled water was used in bottle B in place of Ringer-Locke solution so that laking of the blood readily occurred when 30 to 50 cc. thereof were added. Bottle A on the other hand contained Ringer-Locke solution of 2 to  $2\frac{1}{2}$  times usual strength, so that the total electrolyte content of the mixture of bottles A and B should be about the same as in the red cell suspension experiments.

A number of confirmatory experiments were also done with citrated human red cells suspended in saline; large supplies of these were obtained within 24 to 48 hours of blood donation from the Massachusetts State Antitoxin Laboratory, to whom we are indebted for furnishing this material. In the earlier hemoglobin solution experiments with this material, the blood and CO reagent were each prepared in Ringer-Locke solution of usual strength, the blood cells having been previously laked with 0.2 per cent saponin. This last procedure is less satisfactory, in view of possible action of the saponin on the hemoglobin: nevertheless the results did not differ significantly from those in which no saponin was used. Gasometric estimations were made on 5 cc. samples of fluid B according to the usual Van Slyke procedures.

The concentration of hemoglobin or red cells in the streaming fluid was thus kept within narrow limits, these being so chosen as to give optimal absorption bands for the Reversion Spectroscope measurements. Previous work on sheep's blood showed the kinetic results to be independent of wide variations in the hemoglobin concentration: hence it was not felt necessary to replace the observation tube by others of different diameter, so as to make it feasible, optically, to work with hemoglobin solutions of very different strengths. The concentrations of CO and  $\text{O}_2$  in the mixed fluid were, on the other hand varied 2 to 3-fold, these quantities being known to affect the rates of the reactions observed.

All the Reversion Spectroscope determinations were made by myself, but to reduce the subjective element as far as possible, the actual readings I made were noted down by my colleague, Mr. F. Sargent, without my knowing what they were. He also regulated the driving pressure applied to bottles A and B, turned the reagents on and off for each observation and helped in other ways in the general conduct of the experiments. I am much indebted to him for this assistance.

EXPERIMENTAL RESULTS AND CALCULATIONS. A: *The kinetics of the reaction*  $CO + O_2Hb \rightleftharpoons O_2 + COHb$ . In the case of the earlier experiments on sheep's blood solution the results were found to be consistent with the equation

$$\frac{d[COHb]}{dt} = \frac{m'[CO][O_2Hb]}{[O_2]} - m[COHb]$$

The theoretical basis for this equation is discussed in my previous paper (10). Over the first half of the reaction, the back reaction velocity term,  $-m[COHb]$ , is negligible, so that the equation reduces to:

$$\frac{d[COHb]}{dt} = \frac{m'[CO][O_2Hb]}{[O_2]} \quad (1)$$

Let  $\alpha$  = the total concentration of CO in the mixed fluid in millimols/liter  
 = concentration of dissolved CO + concentration of combined CO  
 =  $[CO] + [COHb]$   
 $\beta$  = total concentration of hemoglobin in the mixed fluid in all forms  
 =  $[COHb] + [O_2Hb] + [Hb]$   
 $\gamma$  = total concentration of  $O_2$  in the mixed fluid  
 =  $[O_2] + [O_2Hb]$   
 $y$  =  $[COHb]$

Then equation (1) becomes

$$\frac{dy}{dt} = \frac{m'(\alpha - y)(\beta - y)}{(\gamma + y - \beta)} \quad (2)$$

$[Hb]$  being neglected because at the concentrations of  $O_2$  used in the experiments it is throughout very small compared with  $\beta$ . Equation (2) when integrated over the time interval  $(t_1 - t_2)$  gives

$$m'(t_1 - t_2) = \frac{\beta - \alpha - \gamma}{\alpha - \beta} \ln \frac{\alpha - y_1}{\alpha - y_2} + \frac{\gamma}{\alpha - \beta} \ln \frac{\beta - y_1}{\beta - y_2} \quad (3)$$

Table 1 shows the application of this equation to a typical experiment on human laked blood solution at 37°C. It is clear that the data of this experiment are fitted adequately by equations (2) and (3). Actually the possible errors in the reversion spectroscope readings and in the other estimations limit the accuracy of the determination of  $m'$  to  $\pm 10$  per cent as a rule, though occasionally divergencies up to twice this amount are found.

Table 2 summarizes the results of eight successful experiments on human red

cells and blood solutions, all at  $37^\circ\text{C}$ . but with varying concentrations of  $\text{CO}$  and  $\text{O}_2$ . The results for the red cell suspensions are also calculated on the assumption

TABLE 1

*Rate of reaction of  $\text{CO} + \text{O}_2\text{Hb} \rightarrow \text{O}_2 + \text{COHb}$*   
 $\alpha = 0.129 \text{ mM.}, \beta = 0.075 \text{ mM.}, \gamma = 0.745 \text{ mM.}$

TIME	%COHb	$\alpha - \gamma$	$\beta - \gamma$	CALCULATION OF $m'$ OVER VARIOUS INTERVALS
<i>seconds</i>				
0	0	0.129	0.075	From $t = 0$ to $t = 0.08$ , $m' = 20.0$
0.08	24	0.111	0.057	From $t = 0$ to $t = 0.14$ , $m' = 23.6$
0.14	40	0.101	0.045	From $t = 0$ to $t = 0.22$ , $m' = 22.6$
0.22	53	0.089	0.035	
Average .....				$= 22.1$

TABLE 2

*Measurements of the velocity constant  $m'$  in human red cells and blood solution at  $37^\circ\text{C}$ ., with varying total  $[\text{CO}]$  and  $[\text{O}_2]$*

TOTAL $[\text{CO}]$	TOTAL $[\text{O}_2]$	RATIO $[\text{CO}]$ TO $[\text{O}_2]$	$m'$
Blood solution experiments			
<i>mM./liter</i>	<i>mM./liter</i>		
0.129	0.745	1.73	22.1
0.34	0.495	0.69	17.2
0.213	0.65	0.33	23.8
Average for blood solutions .....			21.0
Red cell suspension experiments			
0.129	0.314	0.41	20.7*
0.183	0.615	0.30	19.9
0.195	0.664	0.29	23.2
0.105	0.567	0.185	20.3
0.107	0.781	0.137	26.2
Average for red cell suspensions .....			22.0

\* The actual figure calculated from equation (3) was 18.8 but an addition of 10 per cent to this was made to allow for the influence of diffusion through the substance of the red cell. This factor becomes important as the  $[\text{O}_2]$  is lowered below 0.6 mM. The correction factor of 10 per cent is based on the results given in table 3 and the Discussion (q.v.).

tion that equations (2) and (3) are applicable. The results with different blood samples diverged by less than the experimental error of the method, so no separation of the results on the basis of the individual bloods used has been made

in table 2. In five of these experiments the agreement with the mean values is to within  $\pm 10$  per cent: in the other three the discrepancies are +13 per cent, +18 per cent and -18 per cent, so that on the whole the concordance of the results with equation (3) is about as good as could be expected from the accuracy of the method. The changes in total [CO] and total [O<sub>2</sub>] are seen to be without significant effect. The mean value of  $m'$  for human hemoglobin solution agrees to within 5 per cent with that for the red cells at the high [O<sub>2</sub>] concentrations used. The significance of this latter fact, and the circumstances under which such a concordance would not be expected, will be considered later in the Discussion.

B. *The kinetics of the reverse reaction*  $O_2 + COHb \rightarrow CO + O_2Hb$ . The rate of this reverse reaction was previously found to be expressible by the equation

$$\frac{d[COHb]}{dt} = -m[COHb] \quad (4)$$

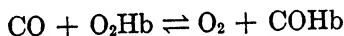
Integration over the time interval  $t_1$  to  $t_2$  gives

$$m(t_1 - t_2) = \ln \frac{[COHb]_1}{[COHb]_2} \quad (5)$$

The theoretical basis of this equation was also discussed in the earlier papers (10). In several tests it was furthermore verified in the case of buffered sheep hemoglobin solution at 15°C. that

$$\frac{m'}{m} = M = [O_2][COHb]/[CO][O_2Hb] \quad (6)$$

where  $M$  is the equilibrium constant of the reaction



It is reasonable to assume that equation (6) must also apply both to human hemoglobin solutions and to red cell suspensions at 37°C. since: (a) several investigators (2, 3) have shown that the equilibrium of the reaction  $CO + O_2Hb \rightleftharpoons O_2 + COHb$  conforms to equation (6) in the case of human blood at body temperature and that the value of  $M$  is the same for whole as for laked blood, and (b) the reaction  $O_2 + COHb \rightarrow CO + O_2Hb$  has been found to proceed at the same rate in the red cell as in solution, in the case of sheep blood at 15°C. (10). There is no ground for supposing that change of species and temperature should alter this concordance in rates.

The best value for  $M$  in human blood at 37 to 38°C. is that given by Sendroy, Liu and Van Slyke (12), namely, 210. These authors found no individual variations within the species in  $M$  for either human blood or ox blood, exceeding the limits of experimental error.

According then to equation (6)

$$m = m'/M = 21/210 = 0.1 \text{ for human hemoglobin and red cells at } 37^\circ\text{C.}$$

From equation (6) the time for half dissociation of human hemoglobin at 37°C.

$$\text{in solution or in the red cell} = \frac{\ln 2}{m} = 6.9 \text{ secs.}$$

To verify these results experimentally would require check estimations of  $M$ , together with determinations of  $m$  at  $37^\circ\text{C}$ . The measurement of  $M$  by the modern gasometric technique is easy and accurate: no very satisfactory procedure is, however, available at present for determining  $m$  at  $37^\circ\text{C}$ . Of the two methods used previously (10) for  $m$ , one—the shaking method—is impracticable at  $37^\circ\text{C}$ ., whereas the other—the rapid flow method—has been found to give only very rough results under these conditions. This is one reason why the required tests have not yet been made; another is that the indirect type of calculation of  $m$  by equation (6) has proved so reliable in the case of other hemoglobin reactions (7) that there can be little doubt of its validity in the present case.

C. *Rough determination of rate of uptake of  $\text{O}_2$  by reduced human red cell suspensions at  $37^\circ\text{C}$ .* An idea of the order of magnitude of the speed with which reduced (i.e.,  $\text{O}_2$ -free) human red cell suspensions take up  $\text{O}_2$  at  $37^\circ\text{C}$ . was required for several purposes, of which one, the estimate of a minimum value of the diffusion constant of the red cell membrane to  $\text{O}_2$  or  $\text{CO}$ , is made use of in the Discussion. It was obtained as follows: 150 cc. of warm Ringer-Locke solution (1.2 times normal strength) were equilibrated in a 300 cc. tonometer with oxygen, by shaking the tonometer in a water bath at  $39.5^\circ\text{C}$ . In a second tonometer, 150 cc. of similar warm Ringer-Locke solution were first deaerated by repeated evacuation and shaking; 15 cc. of freshly drawn human heparinized blood were similarly deaerated in a separate tonometer, and 10 cc. thereof were transferred therefrom by means of a syringe to the deaerated Ringer-Locke solution, with which it was completely mixed. The two tonometers were then joined up to either side of a 2 mm. bore T-tap, and the two fluids driven into the mixing space of the tap by means of compressed air of  $\frac{1}{2}$  atmosphere pressure. The mixed fluid emerging therefrom and streaming down the observation tube showed a color gradient ranging from the purple color of reduced hemoglobin (at the beginning of the observation tube) to the scarlet of oxyhemoglobin. The distance to which the color gradient extended up the tube was roughly measured by eye and was found to be  $5 \pm 1$  cm., at a rate of flow of 240 cm./sec. Thus the time to reach about 90 per cent  $\text{O}_2\text{Hb}$  in the red cells =  $0.02 \pm 0.004$  sec. with an average  $p\text{O}_2 = \frac{1}{2}$  [330 mm. Hg (initial pressure of  $\text{O}_2$  in mixed fluid surrounding cells) + 110 mm. Hg (final pressure of  $\text{O}_2$  in mixed fluid surrounding cells)] = 210 mm. Hg. The process observed depends upon the speed of three processes:  $a$ , the diffusion of  $\text{O}_2$  through the red cell membrane;  $b$ , the diffusion of  $\text{O}_2$  through the substance of the red cell;  $c$ , the combination of  $\text{O}_2$  with hemoglobin in the cell. A minimum value for the speed of process  $a$  is obtained by assuming that processes  $b$  and  $c$  are instantaneous in comparison therewith. On this basis, the minimum value for the diffusion constant of the red cell membrane (for red cells suspended in Ringer-Locke solution) is such as to give an increase of per cent  $\text{O}_2\text{Hb}$  in the cell =  $90/(210 \times 0.02) = 21.5$  per cent  $\text{O}_2\text{Hb}$  per sec. per mm. Hg gradient of partial pressure of  $\text{O}_2$  across the red cell membrane. For  $\text{CO}$  the corresponding figure would probably be about 15 per cent lower, i.e., 18.0, assuming the same ratio of  $\text{CO}$  and  $\text{O}_2$  diffusion constants through the membrane as for the diffusion constants of  $\text{O}_2$  and  $\text{CO}$  in



water. The actual values, according to the suggestions of previous work (9), are probably not less than twice this minimum figure. I hope that it will later be possible, with the aid of the improvements in the rapid reaction velocity methods introduced by Chance (1) and Millikan (7), to obtain much more accurate estimates.

**DISCUSSION.** *The significance of the concordance in rates of the  $\text{CO} + \text{O}_2\text{Hb} \rightarrow \text{O}_2 + \text{COHb}$  reaction in hemoglobin solution and in the red cell.* In solution the reaction is a straightforward homogeneous process, dependent for its rate only on the concentrations of the reagents and the size of the velocity constants. In the red cell four other processes are also involved: *a*, diffusion of CO inwards through the red cell membrane; *b*, diffusion of CO through the substance of the red cell; *c*, diffusion of  $\text{O}_2$  through the substance of the red cell; *d*, diffusion of  $\text{O}_2$  outwards through the red cell membrane. The question now to be discussed is whether these processes are all so fast as compared with the speed of the primary chemical reaction, that the concordance in rates in solution and in the red cell must be due to the primary reaction rate being the same in solution as in the red cell and being the limiting factor in the latter case.

Processes *a* and *d* can be handled by means of the minimum value of the diffusion constant of the red cell membrane, which has just been calculated. Let us, as an example, take the last experiment given in table 2.

The maximum rate of decrease of the red cell per cent  $\text{O}_2\text{Hb}$  in this case = 300 per cent per sec.

The maximum difference of  $\text{O}_2$  pressure ( $\Delta p\text{O}_2$ ) required across the membrane to maintain this rate =  $300/21.5 = 14$  mm. Hg, which is only 2.8 per cent of the average  $\text{O}_2$  pressure in the suspension fluid ( $p\text{O}_2$  corresponding to the initial concentration of dissolved  $\text{O}_2$  of  $0.706$  mM.  $[\text{O}_2]$  at  $37^\circ\text{C.} = 500$  mm. Hg).

Similarly the maximum difference of partial pressure of CO ( $\Delta p\text{CO}$ ) across the membrane =  $300/(18) = 17$  mm. Hg which is 14 per cent of the average CO pressure of 122 mm. Hg in the suspension fluid at the beginning of the experiment. This second figure is itself appreciable but it may be as much as twice too high if the assumed minimal values for the diffusion constant of the red cell membrane are, as suggested above, only half or so of their true values. It seems fair to conclude that the influence of diffusion through the red cell membrane should be practically within the limits of error in determining  $m'$ , provided the  $[\text{O}_2]$  exceeds  $0.6$  mM. At lower values of  $[\text{O}_2]$  the rate of the primary reaction (i.e.,  $m'[\text{CO}][\text{O}_2\text{Hb}]/[\text{O}_2]$ ) might definitely be large enough to involve an appreciable  $\Delta p\text{CO}$  across the membrane. This is one reason why the  $[\text{O}_2]$  was kept at about  $0.6$  mM or over in most of the red cell suspension experiments so far carried out.

The diffusion of CO and  $\text{O}_2$  inside the substance of the red cell is not really separable from the concurrent chemical reaction  $\text{CO} + \text{O}_2\text{Hb} \rightarrow \text{O}_2 + \text{COHb}$ . Consider for example the state of affairs in the layer of hemoglobin bounded by planes distant  $x$  and  $x + \Delta x$  respectively from the surface of the red cell. At any particular instant of time  $t$  there will exist a certain gradient of CO pressure,  $\Delta p$ , across this layer: this gradient is in part responsible for the reaction of CO with the hemoglobin in the layer during the time interval  $\Delta t$  succeeding  $t$ .

and in part for the diffusion of CO through the layer during  $\Delta t$  to enter parts of the red cell beyond the plane  $x + \Delta x$ . Similar considerations apply to the movement of  $\text{O}_2$  within the red cell. The problem can only be treated theoretically by some adaptation of the methods I used in a previous paper (9) on "Diffusion and chemical reaction velocity as joint factors in determining the rate of uptake of oxygen and carbon monoxide by the red blood corpuscle." In this paper I developed theoretical formulae for the rate of entry of  $\text{O}_2$  or CO into a layer of hemoglobin solution of the same average concentration and thickness as the red cell. The mathematical equations involved both diffusion and chemical reaction velocity constants and were too complicated to solve exactly. I was, however, able to obtain maximum and minimum solutions which were close enough to one another to enable the true solution (which must lie between them) to be reached to within a numerical accuracy of  $\pm 10$  per cent, over a considerable range for each process.

These formulae cannot be directly applied to the present problem since in this case there are *two* gases, CO and  $\text{O}_2$ , diffusing instead of only one: if, however, the  $\text{O}_2$  is present in considerable excess over the CO, as in the last two experiments of table 2, it can be shown that only a small error is made if its concentration is taken as constant. The maximum and minimum rate solutions of my previous paper then become directly applicable.

Table 3 shows a comparison of the rates of CO uptake as calculated in this way by *a*, human hemoglobin solution at  $37^\circ\text{C}$ . in presence of a constant partial  $p\text{CO} = 200$  mm. Hg, and 3 different constant partial pressures of  $\text{O}_2$  covering the range used in table 2; *b*, a layer of human hemoglobin solution at  $37^\circ\text{C}$ . and of the same average concentration and thickness ( $2\mu$ ) as that present in the red cell. In this case the maximum and minimum solutions are given. It will be seen that the maximum and minimum rates are within 14 per cent of each other, and their mean is within 5 per cent of the rate in plain solution in the case of the two higher partial pressures of  $\text{O}_2$ , but a divergence begins to appear as the  $p\text{O}_2$  is lowered and the rates of the chemical reaction increase beyond a certain limit. This result is in general accord with the conclusion of my previous paper (9) that the slower the chemical reaction involved, the greater the tendency for the overall rate in the red cell to agree with the rate in solution, *provided* that the reaction kinetics are the same for the hemoglobin in the concentrated milieu of the red cell as in solution. Conversely the finding of an equality between the rate of the over-all reaction in the red cell and of the simple reaction in solution, is strong evidence that the reaction kinetics are in fact the same in the two cases.

*Note on the reactivity of hemoglobin in solution and in the red cell.* Hill and Wolverkamp (5) have reported a larger difference between the position of the oxyhemoglobin dissociation curve of red cell suspensions and of laked hemoglobin solutions than they feel can be explained by pH effects. The oxyhemoglobin dissociation curve is, however, also affected by other electrolytes besides the  $\text{H}^+$  ion (13) and no comparison is known to me in which the concentrations of all electrolytes inside the red cell have been duplicated in a solution of hemo-

globin. The velocity constants of  $m$ ,  $m'$ , and the equilibrium constant,  $M$ , of the  $\text{CO} + \text{O}_2\text{Hb} \rightleftharpoons \text{O}_2 + \text{COHb}$  are, on the contrary, very little, if at all, affected by pH or electrolytes. The experimental concordance in the values of  $M$ ,  $m$  and  $m'$  in solution with their values in the red cell (provided in the case of  $m'$

TABLE 3

Comparison of calculated rates of the reaction  $\text{CO} + \text{O}_2\text{Hb} \rightleftharpoons \text{O}_2 + \text{COHb}$  in solution and in red cells for human blood at  $37^\circ\text{C}$ .

[CO]	pCO	[O <sub>2</sub> ]	pO <sub>2</sub>	TIME	%COHb IN SOLUTION	%COHb IN RED CELL SUSPENSION			MEAN CELL RATE SOLUTION RATE
						Maximum	Minimum	Mean	
mM./liter	mm. Hg	mM./liter	mm. Hg	secs.					
0.216	200	1.0	710	0.05	19	19	17.4	18.2	0.96
0.216	200	0.6	425	0.05	29.5	29.5	26.5	28.0	0.95
0.216	200	0.3	212	0.025	29.5	26.8	24.2	25.6	0.87
0.216	200	0.3	212	0.050	50.6	50.0	43.0	46.5	0.92
0.216	200	0.15	106	0.0125	29.5	26.5	19.0	22.8	0.77
0.216	200	0.15	106	0.025	50.6	48.8	39.8	44.3	0.87

Assumptions:

- (1) Rate in solution is given by  $\frac{d[\text{COHb}]}{dt} = k'p\text{CO}[\text{O}_2\text{Hb}]$

$$\text{where } k' = \frac{m'}{[\text{O}_2]} \times \frac{[\text{CO}]}{p\text{CO}} = \frac{21 \times 0.00108}{[\text{O}_2]} = \frac{0.0217}{[\text{O}_2]} \text{ at } 37^\circ\text{C}.$$

- (2) Maximum rate is given by inequality (IXc) of my previous paper (9, p. 22)

$$\text{viz. } \sqrt{y_0} - \sqrt{\bar{y}} < \frac{p\text{CO } t}{2b} \sqrt{k'D} \tanh \left( \sqrt{\frac{k'y_0 b}{D}} \right)$$

- (3) Minimum rate is given by inequality (X) (9, p. 25)

$$\text{viz. } y_0 - \bar{y} > \frac{2D}{b^2 k'} \ln \left\{ \cosh \left( b \sqrt{\frac{k'y_0}{D}} \right) / \cosh \left( b e^{-k'p\text{CO}t} \sqrt{\frac{k'y_0}{D}} \right) \right\} - ap\text{CO}$$

where  $y_0$  = initial concentration of  $\text{O}_2\text{Hb}$  in red cell, expressed in cc.  $\text{O}_2$  combined per cc. red cell contents = 0.4

$\bar{y}$  = average value of  $\text{O}_2\text{Hb}$  in red cell at time  $t$  expressed in same units

$2b$  = average thickness of human red cell =  $2\mu = 2 \times 10^{-4}$  cm.

$D$  = diffusion constant of red cell substance to CO

= cc. CO diffusing per sec. through layer of 1 sq. cm. area, 1 cm. thickness under a pressure gradient of 1 mm. Hg across layer =  $4.5 \times 10^{-10}$  at  $37^\circ\text{C}$ . (6)

- (4) Average per cent COHb at time  $t = 100$  - average per cent  $\text{O}_2\text{Hb}$  at time  $t =$

$$100 \left( 1 - \frac{\bar{y}}{y_0} \right)$$

that the  $[\text{O}_2]$  is made high enough for the influence of diffusion in the red cell to be unimportant) therefore suggests that the concentration and state of the hemoglobin in the red cell do not, per se, appreciably change the reactivity of the molecule with  $\text{O}_2$  and CO. Such differences as there are from hemoglobin in solution would seem to be more likely due to the contiguity of the other substances present in the red cell.

*Scope of further experimental work.* The main gap in the present data on red cell suspension is in the lack of sufficient variation in  $[\text{O}_2]$ . For the reaction to conform to the equation  $d[\text{COHb}/dt] = m'[\text{CO}][\text{O}_2\text{Hb}]/[\text{O}_2]$  it is true that the concentration of reduced Hb must be very small compared with that of the  $\text{O}_2\text{Hb}$ . The  $[\text{O}_2]$  could, however, have been reduced to as low as 0.15  $mM$  without the reduced Hb exceeding 2 per cent of the total Hb concentration. At  $[\text{O}_2] = 0.15\text{ }mM$ , table 3 shows that the rate in red cell suspensions should have been 20 per cent or so less than the rate in solution if the diffusion of CO through the substance of the red cell alone be taken into account. When the effects of  $a$ , the diffusion of  $\text{O}_2$  through the substance of the red cell, and  $b$ , the diffusion of both gases through the red cell membrane are added, a difference of perhaps as much as 40 per cent might be expected. This should be experimentally detectable beyond doubt, and it would consequently be very interesting to carry out such a series of experiments. Other factors which should be studied are the effect of temperature and pH, and also the influence of changes of  $[\text{Na}]$  to  $[\text{Ca}]$ , in the suspending fluid and of other factors (e.g., presence of plasma protein) which might be expected to alter the permeability of the red cell membrane. In addition, data on a wider range of individual bloods in different physiological conditions are obviously desirable. The present data at high  $[\text{O}_2]$  are, however, adequate for the physiological applications made in my adjoining paper, provided it is fair to assume that the permeability of the red cell membrane in the circulating blood is not markedly lower than its *in vitro* value in Ringer-Locke solutions at  $37^\circ\text{C}$ .

#### SUMMARY

1. Measurements are given of the velocity with which CO displaces  $\text{O}_2$  from combination with human hemoglobin in solution and in unlaked red cells at  $37^\circ\text{C}$ .

2. The kinetic data conform to the same equation as has been established for sheep hemoglobin solution at 10 to  $20^\circ\text{C}$ ., namely,

$$\frac{d[\text{COHb}]}{dt} = \frac{m'[\text{CO}][\text{O}_2\text{Hb}]}{[\text{O}_2]} - m[\text{COHb}]$$

3. At  $[\text{O}_2]$  values of 0.6  $mM$  and over, the value of  $m'$ , i.e., 21.0, is within error the same for hemoglobin in solution as in the red cell. This indicates that at high  $[\text{O}_2]$  values diffusion through the membrane and substance of the red cell has no limiting effect on the velocity of the overall reaction. At lower  $[\text{O}_2]$  values theoretical calculations suggest that the rate in the red cell would be less than in solution but this has not yet been experimentally verified.

4. The value of  $m$  at  $37^\circ\text{C}$ . is 0.1. The time for half dissociation of human COHb is thus 6.9 seconds in the body.

5. A minimum value for the permeability of the red cell membrane to  $\text{O}_2$  is given.

6. The kinetic data of this paper are available for several physiological problems such as the rate of uptake and output of CO from the human body and the calculation of the average time spent by the blood in the lung capillaries.

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# THE AVERAGE TIME SPENT BY THE BLOOD IN THE HUMAN LUNG CAPILLARY AND ITS RELATION TO THE RATES OF CO UPTAKE AND ELIMINATION IN MAN

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In an adjoining paper (3) on the rate of CO uptake by man under various conditions it was shown that substitution of oxygen for air in CO-containing atmospheres led to a drop in the initial rate of CO uptake, amounting on the average to 23 per cent at rest and to 38 per cent during hard work. This difference was attributed to the slower rate of reaction of CO with hemoglobin at high  $O_2$  pressures, in consequence of which a more appreciable back pressure of CO develops in the blood in the lung capillaries when breathing CO in  $O_2$  than when breathing the same per cent CO in air. In the present paper I propose to make use of this observed difference, together with recent data (14) on the rate of chemical reactions between CO and human  $O_2Hb$  both in solution and in the red cell at  $37^\circ C.$ , in order to calculate approximately the average time spent by the red cell in traversing the capillaries of the lung. Whilst the blood is in this location it is in very intimate contact with the air of the alveoli and the atria of the lungs (the area of the intervening membrane is of the order of 50 to 100 sq. m. and the thickness is of the order of 1 to  $2\ \mu$ ); only here, indeed, are conditions suitable for an adequate exchange of  $O_2$  and  $CO_2$  between the air and the blood to occur by diffusion. In any attempt to work out the detailed kinetics of normal  $O_2$  and/or  $CO_2$  exchange in the lung, it is accordingly necessary to know not only the area and thickness of the alveolar membrane (as expressed by the "Diffusion Constant" (10) of the lung) and the speeds of the chemical processes in the blood, but also the average length of time during each circulatory cycle in which these changes are able to occur.

No data have, to my knowledge, existed heretofore as to the magnitude of this physiologically important time interval. In previous computations of the kinetics of gas exchange between the blood and the alveoli (6, 12) average times of the order of 1 to 5 seconds have usually been assumed, though without any quantitative basis other than the general impression given by microscopic observation of the speed of capillary blood flow and of the length of the capillaries. Numerous estimates have, of course, been made of the total circulation time through the lungs either by 1, division of the total amount of blood estimated to be present in the lungs by the cardiac output per minute; 2, injection of tracer substances into the jugular vein, followed by noting the time of their appearances in the artery. Such methods have given times of the order of 3 seconds in the case of mammals, including man, but there has been no indication as to what fraction of these "total" lung circulation times is represented by the passage through the alveolar capillaries.

The method of calculation now to be described gives an answer, correct probably to about  $\pm 30$  per cent, to this unsolved problem concerning the respiratory processes in the lungs. The figures so obtained are later applied in this paper to a brief consideration of the factors limiting the rate of CO uptake and elimination via the lungs. In future work it is hoped to apply the data to the kinetics of  $O_2$  and  $CO_2$  exchange in the lungs, wherein several questions are still outstanding (see e.g., 13).

PRINCIPLES OF THE METHOD OF CALCULATION. Elsewhere I have shown that the velocity of the reaction  $CO + O_2Hb \rightarrow O_2 + COHb$  for human red cells in vitro, in presence of high concentrations of  $O_2$  at  $37^\circ C$ ., is given by the equation:

$$r = \frac{d[COHb]}{dt} = \frac{-d[O_2Hb]}{dt} = \frac{m'[CO]}{[O_2]} [O_2Hb] = \frac{21[CO]}{[O_2]} [O_2Hb] \quad (1)$$

I assume that this equation applies in vivo in the lung capillaries when a man breathes  $O_2$  containing a low percentage of CO. If so, then

$$\begin{aligned} r_L &= \text{rate of formation of COHb in the lung capillaries} \\ &= \frac{21 \times 0.77 \times \bar{p}CO_L}{\bar{p}O_{2L}} [O_2Hb] \end{aligned} \quad (2)$$

where  $\bar{p}CO_L$  is the average pressure of CO in the lung capillaries

$\bar{p}O_2$  is the average pressure of  $O_2$  in the lung capillaries

and the factor 0.77 is the ratio of the solubility coefficients of CO and  $O_2$  at  $37^\circ C$ .

$\bar{p}O_{2L}$  is calculated from the equation

$$\begin{aligned} &(\text{Average alveolar } pO_2 - \bar{p}O_{2L}) \times \text{Diffusion Constant} \\ &\text{of the lungs to } O_2 = \text{cc. } O_2 \text{ absorbed per minute} \end{aligned} \quad (3)$$

$\bar{p}CO_L$  is calculated to within  $\pm 10$  per cent by the method described below in the illustrative example.

Let  $r_B$  = observed rate of increase of CO in the blood during the  $O_2$ -breathing experiment.

Then

$$\frac{r_B}{r_L} = \frac{t_L}{t_B} \quad (4)$$

where  $t_L$  = average time spent by the blood in the lung capillaries,

$t_B$  = average time of the total circulation,

= 60  $B/C$  seconds,

$B$  = total blood volume in liters,

$C$  = cardiac output in liters per minute.

The only unknown in equation (4) is  $t_L$ , the quantity whose value is desired.

The reason why equation (4) must hold good is that formation of COHb can, and does, only occur to an appreciable extent whilst the blood is actually in the

lung capillaries in close contact with the alveolar air. The excess of CO in physical solution in the blood at the moment the latter leaves the lung capillary will, it is true, nearly all combine with the hemoglobin later, but the actual maximal increase in per cent COHb caused thereby can be shown to be only of the order of 1 to 2 per cent of the increase in per cent COHb which occurs during the transit through the capillary. Thus, in the example presently to be given, the increase in per cent COHb during each passage through the lung capillary works out at about 0.37 per cent. The  $p\text{CO}$  at the moment of leaving the lung capillary is about 0.05 per cent atm. = 0.000009 cc. dissolved CO per cc. of blood, which is chemically equivalent to 0.0045 per cent COHb, i.e., to 1.2 per cent of 0.37 per cent COHb. During the whole of the rest of the circulatory cycle, the rate of formation of COHb is thus effectively nil, so that the rate of increase of COHb in the circulation divided by the rate of increase of COHb in "in vitro" experiments under the same conditions must equal the ratio of the average time spent by the blood in the lung capillaries to the average time of the whole circulation as equation (4) states.

*Calculation of  $t_L$  in a typical example of hard work.* The remaining steps in the method are best seen in working out an actual example, based on the experiment on W. F. at hard work (see table 3 of reference 3), whilst breathing CO, in air on the one hand, and in  $\text{O}_2$  on the other.

The average alveolar per cent CO during the respiratory cycle was obtained as described later. The values were:

0.051 per cent atmosphere for the CO in the air experiment, and  
0.086 per cent atmosphere for the CO in the  $\text{O}_2$  experiment.

The observed rate of increase of COHb = 1.35 per cent per minute for the air experiment and 1.0 per cent for the  $\text{O}_2$  experiment. (Given in table 3, reference 3 in vols. per cent, i.e., as 0.27 and 0.20 respectively.)

The alveolar per cent  $\text{O}_2$ , in the  $\text{O}_2$ -breathing experiment,

= 98 per cent (allowance of 2 per cent for  $\text{N}_2$  impurities)—pressure of  
water vapor in alveolar air—alveolar  $p\text{CO}_2$   
=  $88 \pm 1$  per cent atmosphere

Assuming an  $\text{O}_2$  uptake of 1800 cc. per minute in hard work and a Diffusion Constant of the lungs of 60 we have

$$(88 - \bar{p}\text{O}_{2L}) \times \frac{760 \text{ mm.}}{100} \times 60 = 1800$$

whence  $p\text{O}_{2L} = 84$  per cent atmosphere. To obtain  $\bar{p}\text{CO}_L$  we first find a minimum value and then a maximum value of this quantity. These two extreme values are found to agree to within 20 per cent, and their arithmetic mean cannot therefore diverge by more than  $\pm 10$  per cent from the true value of  $\bar{p}\text{CO}_L$ .

The minimum value of  $\bar{p}\text{CO}_L$  is obtained as follows: The rate of CO uptake



both in the air and the  $O_2$ -breathing experiments must be proportional to the gradient of CO pressure across the lung membrane. Therefore

$$\frac{\text{Rate of CO uptake in } O_2}{\text{Rate of CO uptake in air}} = \frac{1.0}{1.35} \\ = \frac{0.086 \text{ per cent atmosphere} - \text{minimum } \bar{p}CO_L}{0.051 \text{ per cent atmosphere} - \text{zero}} \quad (5)$$

since the average pressure of CO in the lung capillaries in the air experiments cannot be less than zero. From equation (5) minimum value of  $\bar{p}CO_L = 0.048$  per cent atmosphere. The maximum value of  $\bar{p}CO_L$  is obtained as follows:

Let  $y$  = maximum value of  $\bar{p}CO_L$  in the air experiment  
 $z$  = maximum value of  $\bar{p}CO_L$  in the  $O_2$  experiment.

Then by the same argument as in the case of equation (5)

$$\frac{0.086 - z}{0.051 - y} = \frac{1.0}{1.35} \quad (6)$$

The rate of the chemical reaction  $CO + O_2Hb \rightarrow O_2 + COHb$  in the blood of the lung capillaries must also be 1.0/1.35 smaller in the  $O_2$  experiment than in the air experiment. Therefore by equation (2)

$$\frac{21 \times 0.77 \times z}{\bar{p}O_{2L} \text{ in } O_2 \text{ experiment}} \bigg/ \frac{21 \times 0.77 \times y}{\bar{p}O_{2L} \text{ in air experiment}} = 1.0/1.35 \quad (7)$$

The  $\bar{p}O_{2L}$  in the  $O_2$  experiment, as calculated above, is 84 per cent atmosphere. If a greater value for the  $\bar{p}O_{2L}$  in the air experiment is assumed than could have actually existed, it can be seen that the values of  $y$  and  $z$  as given by numerical solution of the simultaneous equations (6) and (7) would both be greater than their true values and would accordingly be maximal values. On this basis the  $\bar{p}O_{2L}$  in the air experiment is taken as 13 per cent atmosphere, this being the  $pO_2$  in the arterial blood leaving the lungs, when breathing air at sea level. This rather difficult point may be explained in another way as follows: Since the rate of reaction of CO with the hemoglobin of the red cells in the lung capillaries is inversely proportional to the  $pO_2$  (according to equation (2)), the CO would be unduly hindered from combining with hemoglobin and would thus build up a higher back pressure in the blood than it actually does, if the value assumed for the  $pO_2$  is greater than the true value. The latter is certainly the case if the arterial  $pO_2$  is used in place of the average  $\bar{p}O_{2L}$  in the air breathing experiment. Equation (7) on this basis reduces to

$$\frac{z}{84} \bigg/ \frac{y}{13} = 1.0/1.35 \quad (8)$$

Solving the simultaneous equations (6) and (8) we find

$$y = 0.012 \text{ per cent atmosphere, } z = 0.057 \text{ per cent atmosphere.}$$

The mean value for  $\bar{p}CO_L$  thus  $= 1/2(0.048 + 0.057) = 0.052$  per cent atmosphere. This mean value, as mentioned above, must certainly be within  $\pm 10$

per cent of the true value. For the  $O_2$ -breathing experiment on W. F. equation (2) then gives initially

$$r_L = \frac{21 \times 0.77 \times 0.052}{84} [O_2Hb] = 1 \text{ per cent COHb per second,}$$

assuming that at the start the  $[O_2Hb] = 100$  per cent (see later).

The observed initial rate of CO uptake by the circulating blood,  $r_B$ , was in this experiment 0.017 per cent COHb per second.

According to Asmussen's data (1) the average cardiac output in liters per minute for this grade of work = 0.22 times the body weight in kgm. (73 for the subject W. F.) = 16 liters per minute. The blood volume by the T-1824 method, was 5.8 liters.

$$t_B = 60 \times 5.8/16 = 21.7 \text{ seconds}$$

Therefore, by equation (4)

$$t_L = t_B \times \frac{r_B}{r_L} = 21.7 \times \frac{0.017}{1.0} = 0.37 \text{ second.}$$

Since  $r_L$  was shown in this experiment to be 1 per cent COHb per second, the blood COHb must increase by 0.37 per cent each time that it passes through the lung capillary (during the early phase in which the uptake rate is linear). It will be recalled that the figure of 0.37 per cent COHb was used earlier in the proof that the lung capillary is the sole seat of COHb formation during CO uptake.

*Data on  $t_L$  at rest and in hard work, their accuracy and relation to other physiological results.* Table 1 summarizes the values of  $t_L$  calculated in the above way from the data given in tables 2 and 3 of the adjoining paper by Forbes, Sargent and Roughton (3). The accuracy of the overall calculation, assuming the theoretical premises on which it is based are correct, depends principally on the numerical accuracy of five factors used in the procedure:

(a) The blood volume—this was measured by the T-1824 dye method. The absolute accuracy should certainly be within  $\pm 10$  per cent.

(b) The cardiac output—the values were not measured but were calculated from the formulae given by Asmussen (1), viz., surface area in square meters  $\times 2.49$  for rest, and weight in kgm.  $\times 0.22$  for a grade of hard work equivalent to that used in our experiments. These assumed values (according to Asmussen's data) probably do not diverge by  $\pm 15$  per cent at the most from the true values.

(c) The velocity constant,  $m'$ , of the reaction  $CO + O_2Hb \rightarrow O_2 + COHb$ —the same value, viz., 21.0, has been assumed for all the subjects in table 1, and is based on the new kinetic data given in my adjoining paper (14), wherein no significant difference was found between the blood of different individuals. The value of 21.0 is probably accurate to  $\pm 10$  per cent.

(d) The average  $pCO$  in the alveolar capillary blood,  $\bar{p}CO_L$ , in the  $O_2$  experiments—the uncertainty in this quantity in the detailed example worked out above was at most about  $\pm 10$  per cent of itself, and the same is true in all other cases so far calculated.

(e) The average alveolar per cent CO—in the case of the experiments in hard work this was calculated by means of Bohr's formula:

$$\begin{aligned} (\text{Volume of tidal air}) \times \text{per cent CO in expired air} = & (\text{Volume of tidal air} - \\ & \text{Dead Space of Lungs}) \times \text{average per cent CO in alveolar air} + \quad (9) \\ & (\text{Dead Space of Lungs}) \times \text{per cent CO in inspired air.} \end{aligned}$$

According to the researches of Krogh and Lindhard (9), in contradistinction to those of Haldane (4) and of Henderson et al. (5), this method of calculating the average composition of the alveolar air during work gives at least as satisfactory an answer as the average of samples taken at different phases of the respiratory

TABLE 1

*Calculations of average time spent by the blood in the lung capillaries,  $t_E$ , for men at rest and in hard work*

SUBJECT	REST				HARD WORK					
	W. F.		F. C.		W. F.		J. E.		F. M.	
	Air	O <sub>2</sub>	Air	O <sub>2</sub>	Air	O <sub>2</sub>	Air	O <sub>2</sub>	Air	O <sub>2</sub>
Average alveolar $p\text{CO}$ (% atm.).....	0.053	0.117	0.057	0.113	0.051	0.086	0.086	0.101	0.112	0.119
Minimum $p\text{CO}_L$ (% atm.).....	0.0	0.077	0.0	0.060	0.0	0.048	0.0	0.050	0.0	0.052
Maximum $p\text{CO}_L$ (% atm.).....	0.0163	0.0875	0.0114	0.071	0.012	0.057	0.016	0.060	0.016	0.062
Assumed $p\text{CO}_L$ (% atm.).....	0.0081	0.082	0.0057	0.066	0.006	0.052	0.008	0.055	0.008	0.057
$r_L$ in % COHb/second.	—	1.64	—	1.23	—	1.0	—	1.06	—	1.08
Blood volume in liters.	5.8		6.8		5.8		5.1		5.7	
Cardiac output in liters/minute.....	4.7		5.2		16.0		16.2		18.5	
$t_B$ in seconds.....	74		78.5		21.7		18.9		18.5	
$r_B$ in % COHb/second.	—	0.0132	—	0.0133	—	0.017	—	0.0183	—	0.0183
$t_L$ in seconds.....	0.61		0.85		0.37		0.33		0.31	
Average $t_L$ in sec- onds.....	0.73 $\pm$ 0.30 (rest)				0.34 $\pm$ 0.1 (hard work)					

cycle, which are technically difficult to obtain and define when the breathing is rapid and deep. More recent work has confirmed the contention of Krogh, and has furthermore supported his view that the increase in dead space during exercise does not amount to more than about 50 per cent of the resting value, as against other claims of a several hundred per cent increase. Actually in hard work the volume of tidal air (1500 to 2000 cc.) is large compared with the dead space, so that the actual value assumed for the dead space in equation (9) has but little effect on the calculated value for the average alveolar per cent CO. Thus in the case of subject W. F. (weight 160 lbs., height 5 ft. 10 in.) the following calculated results are obtained:

Volume of dead space in cc.....	100	200	300	400
Average alveolar $p\text{CO}$ in per cent atm.....	0.055	0.051	0.048	0.043

The actual values assumed for the dead space in the subjects of table 1 were 200 cc. in the case of the medium sized subjects, W. F. and J. E., and 250 cc. in the case of F. C. (weight 200 lbs., height 5 ft. 8½ in.). The uncertainty in the average per cent CO in the alveolar air so obtained is probably not more than  $\pm 10$  per cent of itself. The same is true of the values used in the resting experiments, which were based on directly measured Haldane-Priestley alveolar samples.

Other factors which enter into the calculation of  $t_L$  are the observed rates of CO uptake and the average  $pO_2$  in the alveolar capillary blood in the  $O_2$  experiments. The possible errors in these quantities are, however, much smaller, i.e., only 2 to 3 per cent.

The maximum error to which the calculated value of  $t_L$  would be subject, if all the errors in the quantities on which it depends happened each to be maximal and in the same direction, mounts up to  $\pm 60$  per cent. It is much more likely, however, that some balancing of sub-maximal errors occurs, and on this account it seems conservative to assume an overall error, at most, of half the above amount, i.e.,  $\pm 30$  per cent, for  $t_L$  in work and a rather poorer accuracy for  $t_L$  in rest, owing in the latter case to the smaller difference between the observed CO uptakes in the air and  $O_2$  experiments respectively. On this basis, the average figures for  $t_L$  in table 1 are given as  $0.75 \pm 0.30$  second at rest, and  $0.34 \pm 0.10$  second in hard work.

The possibility of theoretical sources of error in the calculation of  $t_L$  must now be discussed. In computing the value of  $r_L$ , the rate of COHb-formation in the lung capillary whilst breathing CO in  $O_2$ , it was assumed that the amount of reduced hemoglobin in the red cell was negligible throughout the whole capillary even at the very beginning. Such, however, is not the case, for the mixed venous blood, when breathing  $O_2$ , contains at most 75 to 80 per cent oxyhemoglobin, and therefore when the blood enters the lung capillary 20 to 25 per cent of the hemoglobin is in the reduced form and hence is capable of reacting with CO far more quickly (11) than equation (2) indicates. Neglect of this point would make the calculated value of  $t_L$  too high, but in actual fact the error is insignificant owing to the size of the gradient of  $pO_2$  from the alveoli to the blood at the beginning of the capillary (ca. 80 per cent atm.) as compared with the size of the CO gradient (ca. 0.05 per cent atm.). Assuming an  $O_2$  Diffusion Constant for the whole lung of 40, and an initial  $pO_2$  gradient at rest of 86 per cent (alveolar  $pO_2$ ) less 5 per cent (average  $pO_2$  in mixed blood) = 81 per cent atm., it follows that the initial rate of uptake of  $O_2$  will be at the rate of  $(81 \times 760 \times 40)/100$ , or of 24,600 cc.  $O_2$  per minute, which is about 100 times the average rate at rest. This means that the red cells, when  $O_2$  is breathed, must become practically 100 per cent saturated with  $O_2$  by the time they have proceeded along the first one-hundredth of the lung capillary. Only during such time (i.e., of the order of 0.005 sec.) is appreciable reduced hemoglobin available in the cell to combine directly with the CO. Under the resting conditions given in table 1, the per cent COHb which could be formed in this time by direct combination of CO with Hb can be calculated to be about 0.006 per cent, which is only of the order of one-fiftieth of that formed according to equation (2) in the remainder of the capillary, as indicated by the previous calculations and results of table 1. A

similar result is found in the conditions prevailing during hard work, and it may thus be concluded that neglect of the reduced hemoglobin present in the red cell at the beginning of the capillary does not cause the calculated value of  $t_L$  to be appreciably too high.

A more general point of possible criticism is that the whole method of computation hangs on the assumption that the differences in rate of CO uptake in the air and the O<sub>2</sub> experiments are *entirely* due to the much greater back pressure of CO ( $\bar{p}\text{CO}_L$ ) in the blood in the latter instance. It has been tacitly assumed that there are no significant differences in blood volume, cardiac output, conditions of circulation in the lungs, permeability of the lung membrane, etc., when O<sub>2</sub> is substituted for air whether at rest or in hard work. It has also been assumed that the rate of displacement of O<sub>2</sub> from hemoglobin in the red cell in vivo is the same as in the vitro experiments at 37°C. in my adjoining paper (14). Although it must be admitted that there is no rigorous certainty that such assumptions are correct, it seems reasonable to accept them and the consequences which flow from them, unless and until definite evidence to the contrary is brought forward.

The best way of checking the correctness of the values calculated for  $t_L$  would be to compare them with the results obtained by some more direct method. Dr. J. T. Wearn (private communication) has pointed out to me that it might well be possible from photographs of the circulation in the lungs of animals to make a direct estimate of the value of  $t_L$  in the resting animal. It should also not be too difficult, in a well-trained animal, to make the observations necessary for the calculation of  $t_L$  by the indirect method given in this paper, since this only involves subjecting the animal to known CO-air and CO-O<sub>2</sub> mixtures, measuring the blood volume and cardiac output, and collecting blood samples for the measurement of CO content and of  $m'$ . An agreement between direct and indirect tests of this kind would furnish a good confirmation of the validity of my indirect method for man, in the case of whom a direct test of the type proposed by Doctor Wearn would not presumably be feasible. That the values so far obtained are, however, reasonable is shown by a consideration of their relation to certain other physiological data, in the course of which several quite interesting points will be found to emerge.

(a) The resting value of  $t_L$  appears to agree quite closely with the average time spent by the blood in the intestinal capillaries of the dog, in the example quoted by Bazett (2). The average length of these capillaries is given as 0.4 mm., and the average rate of blood flow as 0.5 mm. per second, leading to an average time of 0.8 second as compared with the figure of 0.75 second in the resting human lung.

(b) It is easy to see that the total volume of blood present in the patent lung capillaries must equal the product of the total volume of blood present in the body and the fraction  $t_L/t_B$ . From the data of table 1 it therefore follows that the average volume of blood present in the lung capillaries amounts to 60 cc. at rest and 95 cc. in hard work. This increase of only about 60 per cent in hard work indicates that the hyper-ventilation entailed thereby does not apparently

lead to the opening up of many new capillaries, a conclusion already suggested by Krogh's measurements of the CO Diffusion Constant of the whole lung, which likewise showed only an increase of about 60 per cent in hard work.

If  $n$  is the number of patent alveolar capillaries,  $l$  their average length, and  $r$  their radius, then

$$nl\pi r^2 = 60 \text{ cc. at rest and } 95 \text{ cc. in hard work.}$$

Assuming with Krogh (8) that  $r$  is the same as the radius of the red cell =  $3.5 \times 10^{-4}$  cm. it follows that the total length of all the lung capillaries,  $nl$ , =  $1.56 \times 10^8$  cm. = 970 miles at rest, and  $2.48 \times 10^8$  cm. = 1540 miles in hard work. The total area of the walls of the lung capillaries =  $2nl\pi r = 3.8 \times 10^5$  sq. cm. = 38 sq. m. at rest, and  $6 \times 10^5$  sq. cm. = 60 sq. m. in hard work.

The total area of the alveolar membrane has been estimated from microscopic measurement to be of the order of 50 to 100 sq. m. (17) in man, and of this one-half to three-quarters is probably occupied by capillaries. The above figures for the total area of the lung capillaries thus appear to be in the same range as the values indicated by histological measurements, and to confirm previous views that the blood in the lung capillaries is practically completely surrounded by alveolar air, with nothing in between save for the very thin layers of flat-celled epithelium. The thickness of the endothelium of the capillary vessels themselves is probably 0.8 to 1.0  $\mu$  (8), and of the alveolar epithelium about 1  $\mu$ , though there is some doubt amongst histologists as to the existence of this second layer (17). Assuming, as a compromise, that the average thickness of the membrane separating the capillary blood from the air in the alveoli is 1.4  $\mu$ , the Diffusion Constant of the whole lung to CO should be roughly equal to  $2nl\pi r d_{co}/1.4 \times 10^{-4}$  cm., where  $d_{co}$  is the volume of CO in cc. which diffuses through 1 sq. cm. of alveolar membrane per minute with a  $pCO$  gradient of 1 mm. Hg per cm. thickness of membrane.

Krogh's (7) value for  $d_{co}$  for connective tissue at 38°C. is about  $1.8 \times 10^{-8}$ . Assuming that this value applies to the alveolar and capillary epithelium, the Diffusion Constant of the whole lung membrane should be of the order of

$$3.8 \times 10^5 \times 1.8 \times 10^{-8} / (1.4 \times 10^{-4}) = 49 \text{ cc. at rest per minute per mm. Hg gradient of CO, and}$$

$$6.0 \times 10^5 \times 1.8 \times 10^{-8} / (1.4 \times 10^{-4}) = 77 \text{ cc. in hard work.}$$

These values are of the same order of magnitude as those given by Krogh in direct "in vivo" determinations of the Diffusion Constant of the human lungs to CO. Krogh's (10) figures range from 25 to 50 at rest and 30 to 70 in hard work.

(c) Roughton et. al. (15), in their study of the effect of sulfanilamide on the rate of output of  $CO_2$  by man during hard work, noted that the reduction in rate was somewhat greater than would have been expected from the inhibitory action of the levels of sulfanilamide present in the blood (3 to 4 mgm. per cent) on the carbonic anhydrase system in the red cells. In their calculations they assumed a  $t_L$  value of the order of 1 to 1.5 seconds. With a  $t_L$  value of 0.34 second, as

found for hard work in this paper, calculations of the type made by them point to a 30 to 40 per cent reduction in the  $\text{CO}_2$  which could be eliminated from the bicarbonate- $\text{CO}_2$  transport system of the blood in the lung capillaries. Assuming that the liberation of  $\text{CO}_2$  from carbamino combination with hemoglobin was unaffected by the sulfanilamide, and remained at 30 per cent of the total  $\text{CO}_2$  liberated in the lung under normal conditions, the net decrease in  $\text{CO}_2$  output in the work experiments with sulfanilamide should have been of the order of 20 to 25 per cent as compared with the  $\text{CO}_2$  output in the same degree of work without sulfanilamide. The observed average decrease in  $\text{CO}_2$  output in four subjects was 15 per cent. Such a test as this is even rougher than those just given under headings (a) and (b), but, as far as it goes, it agrees with them in giving some support to the reasonableness of the  $t_L$  values for hard work obtained above.

In regard to the general problem of the kinetics of  $\text{CO}_2$  transport the values of  $t_L$ , as calculated in this paper both for rest and hard work, are distinctly lower than those used by Henriques (6) and myself (12) in our theoretical treatments. A revision of this work is therefore required, but this should best be deferred until further necessary data on certain other aspects of the problem are available. Of these the most important are quantitative determinations of the actual activity of the carbonic anhydrase in the normal red cell, for which methods are now in sight. Previously the magnitude of this activity has only been guessed at by rather insecure extrapolation methods, though its existence has been demonstrated qualitatively in the intact red cell beyond all doubt.

*Factors affecting the relation of  $t_L$  to the rates of CO uptake and elimination in man.*

(a) *CO uptake.* In an adjoining paper (3) Forbes, Sargent and Roughton have confirmed and extended the work of previous observers on the factors which affect the rate of CO uptake in man when exposed to atmospheres containing CO. They found that the rate varied in different subjects, but in any given individual was proportional initially to the pressure of CO and increased with the ventilation rate though at a slower rate. The calculations summarized in table 1 show that in air the average back pressure of CO in the blood is only of the order of 10 per cent of the alveolar pressure of CO (whether in rest or in hard work) and is therefore apparently not an appreciable factor in limiting the rate of uptake of CO. This conclusion is confirmed by the finding (3) that the rate of CO uptake from air containing a given  $p\text{CO}$  is in light activity the same at lowered  $p\text{O}_2$  (10 to 12 per cent atmosphere) as in air at sea level, provided an allowance is made for the increase in ventilation rate caused by the hypoxia in the former case. At lowered  $p\text{O}_2$  there is much more reduced hemoglobin present in the blood over the whole length of the lung capillary, and therefore the mopping up by hemoglobin of the CO, which has passed through the capillary membrane into the blood, must be correspondingly faster at lowered  $p\text{O}_2$  than at sea level and normal  $p\text{O}_2$ . Since the overall rate of CO is nevertheless the same, the rate of chemical combination of CO in the body must be in both cases fast enough to prevent the development of an appreciable back pressure of CO in the blood.

This is not the case when CO in O<sub>2</sub> is breathed, for in such experiments table 1 shows that the average back pressure of CO in the lung capillaries amounts to more than half the alveolar pressure of CO, and is therefore most decidedly a limiting factor.

Krogh's determinations of the Diffusion Constant of the human lung (10) involve measuring the uptake of CO from the alveoli into the blood while the breath is held during a known short interval (of the order of 10 sec.) and then calculating the volume of CO passing through the membrane per minute per mm. of *p*CO gradient, on the assumption that the back pressure of CO in the blood is negligible. If the latter is not so, Krogh's values would be too low, and in this way an explanation might be available of the discrepancy between the amounts of O<sub>2</sub> absorbed by man when breathing low O<sub>2</sub> pressures in hard work, and the amounts which should diffuse through the lung membrane on the basis of Krogh's Diffusion Constant figures (13). It would be very difficult to calculate with any certainty the size of the back pressure of CO, which might arise in the blood under the conditions of Krogh's experiments, unless resort were made to the device of the present papers, namely, of doing additional Diffusion Constant determinations with varying pressures of O<sub>2</sub> in the lungs. Thus by breathing pure O<sub>2</sub> for some minutes the alveolar *p*O<sub>2</sub> would be brought up to 90 per cent atm. instead of 14 per cent atm. as when breathing ordinary air, and the back pressure of CO in the lung capillaries during the Krogh determination might then become very appreciable (just as in the experiments used in table 1). In that case the value of the Krogh Diffusion Constant should come out lower than when breathing air, and by plotting the values of the Diffusion Constant at a series of different alveolar O<sub>2</sub> pressures, it might be possible, by extrapolation, to arrive at the true Diffusion Constant of the lung with zero back pressure of CO in the blood. It is hoped it may be possible soon to carry out such experiments.

(b) *CO output.* In resting man the rate of elimination of CO from the blood after an exposure to the gas is such that the CO content drops at a logarithmic rate when plotted against time, 250 minutes being the average time for the per cent COHb to fall to half its value (16) when breathing air at sea level. The speed of this process is conditioned by several factors: (a) the rate of removal of CO from the lung alveoli by ventilation with fresh air, (b) diffusion of CO through the lung membrane, (c) the rate of chemical dissociation of COHb in the red cell, (d) the possible disappearance of CO by other channels than the lungs, some evidence for which has been recently found by Root and myself. In the present section the rôle of factor (c) in the lung elimination of CO will be chiefly discussed, use being made of the new data on the values of *t<sub>L</sub>* and on the rate of dissociation of COHb in the human red cell at 37°C. which, according to equation (4) of my adjoining paper (14) is given by

$$\frac{d[\text{COHb}]}{dt} = -m[\text{COHb}] = -0.1[\text{COHb}]$$

whence over the time interval, *t*<sub>1</sub> - *t*<sub>2</sub>,  $\ln[\text{COHb}]_1/[\text{COHb}]_2 = m(t_1 - t_2)$ . The time for half dissociation is thus  $\ln 2/0.1 = 6.9$  seconds.



This time is only of the order of one two-thousandth of 250 minutes, the observed time for half elimination of CO from the blood of man at rest (16) when breathing air. At first sight it might therefore appear that factor (c) is not at all a limiting factor in determining the rate of disappearance of CO from the human blood through the lungs. Actually, however, it is again only whilst the blood is in the lung capillaries themselves that the chemical dissociation of COHb can furnish dissolved CO for diffusion out into the expired air (the argument is the same as in the case of CO uptake treated above).

The minimum time for half elimination of CO through the lungs in vivo is therefore not 6.9 seconds but  $6.9 \times t_B$  (average time of whole circulation)/ $t_L$  (average time in lung capillaries) or 690 seconds at rest. This time is about 5 per cent of the observed rate of half elimination of CO in air at rest, and on this basis the rate of chemical dissociation of COHb in the human red cell in vivo has an influence also of about 5 per cent in determining the rate of CO elimination via the lungs in resting man breathing air.

If O<sub>2</sub> is substituted for air the subject derives an immediate benefit from the extra 2 volumes per cent of O<sub>2</sub> dissolved in his blood and, furthermore, the half time of CO elimination is cut to about 40 minutes, according to unpublished experiments of Root and myself. In this case the rate of chemical dissociation of COHb exerts an influence of about 29 per cent (i.e.,  $(100 \times 690)/(40 \times 60)$ ). Better still than pure O<sub>2</sub> is the widely used carbox mixture of 5 to 7 per cent CO<sub>2</sub> in O<sub>2</sub>: the hyperventilation and fall of blood pH produced thereby lead to a further, and quite marked, increase in rate of CO elimination and do, indeed, probably bring the latter much nearer to the limiting half time of 690 seconds, dictated by the velocity constant,  $m$ , of the chemical dissociation of COHb at body temperature. These figures indicate strikingly the practical value of the principles on which the modern technique of resuscitation from CO poisoning is based: they also show, however, that there is little hope of increasing still further the rate of CO elimination via the lungs unless some method can be found of actually changing the value of  $m$  in vivo. Of this the prospect seems poor, for, in vitro,  $m$  is scarcely affected by pH, or by salts, though it is believed to have a rather high temperature coefficient, and is known to be much accelerated by light. Large changes by either of these means do not, however, seem feasible in the actual human lung circulation, and it would seem that any further efforts to increase greatly the rate of disappearance of CO from the blood after poisoning would have to be in the direction of accelerating the loss of CO by other channels than the lungs, assuming that further work confirms the existence of such channels.

In the working subject the theoretical maximum rate of CO elimination in the lungs is rather higher than at rest, since the value of  $t_L/t_B$  averages 0.017 instead of 0.01.

#### SUMMARY

1. The average time,  $t_L$ , which the blood spends in passing through the lung capillaries is of interest, since it is only during this phase of the circulatory

cycle that exchange of gases between the blood and air is possible. A knowledge of the value of  $t_L$  is therefore important in several kinetic problems of respiration.

2. A theoretical method of calculating  $t_L$  is described and its reliability critically discussed. Its accuracy in normal man is believed to be of the order of  $\pm 30$  per cent.

3. The average value is  $0.75 \pm 0.25$  second for normal men at rest, and  $0.34 \pm 0.1$  second in hard work. These values are of the order of magnitude to be expected from other physiological data.

4. The total volume of the blood in the patent lung capillaries is also calculated from  $t_L$ . In normal men it averages 60 cc. at rest and 95 cc. in hard work, thus indicating that no very extensive opening up of extra capillaries occurs in the lungs during exercise.

5. Applications of the new data to the kinetics of CO uptake and elimination in the lungs of man are discussed.

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## TOLERANCE TO ARSENIC TRIOXIDE IN THE ALBINO RAT

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The fact that some of the natives of Styria, a province of Austria, ingest large quantities of arsenic trioxide with supposedly beneficial effects, has led to considerable discussion and some investigation as to the development of tolerance to the drug.

Arsenic eaters start with small doses of arsenic trioxide and gradually increase the quantity ingested until it greatly exceeds that which would be poisonous to the normal individual. However, the ability of the individual or experimental animal to acquire a true tolerance to arsenic trioxide, either solid or in solution as sodium arsenite, has been questioned by several investigators. Schwartz and Munch (1, 2) came to the conclusion that all cases could be explained on the basis of the slow rate of solution of arsenic trioxide, as they found that the toxicity of arsenic trioxide depended largely upon the physical state of the solid. They were unable to show any acquired tolerance in cats to arsenic in solution.

Issekutz and Vegh (3) found that dogs rendered tolerant to solid arsenic trioxide would succumb to relatively smaller doses when administered in solution and that dogs cannot be rendered tolerant to solutions of arsenic. They believed that tolerance is not due to an acquired immunity of the intestinal wall but that it is referable to the fact that the arsenic trioxide is not absorbed, simply because it is not dissolved.

In the course of studies on the detoxification of arsenic by rats in this laboratory, it was desired to administer the largest daily doses that could be safely given. Solutions of sodium arsenite were injected intraperitoneally. However when a group of three-month-old rats had been injected with sublethal doses for about three weeks it was found that the dose could be increased to a level which invariably killed normal animals, without producing toxic effects.

Although the literature led to the opinion that a systemic tolerance to arsenic trioxide or sodium arsenite could not be produced we were prompted to reinvestigate that possibility.

**EXPERIMENTAL.** In the first experiment in which tolerance was noted, five three-month-old female albino rats (group 1) on a diet of purina "checkers" and tap water were subjected to almost daily intraperitoneal injections of 4 to 8

mgm. per kilogram of arsenic trioxide, given as a solution of sodium arsenite (pH 7.2-7.6). At this dose level it had been previously observed that rats in this age group maintained their weight and showed no sign of arsenic poisoning even after six weeks of treatment. After almost three weeks of injection at 4 to 8 mgm. per kgm. level the dose was raised to 10 and, in some cases, to 12 mgm. per kgm. At this higher dose level one animal died after nine daily injections, two survived for one month and two survived for more than fifty days. Control animals, of the same age, which had received no previous injections of arsenic trioxide, died after a single dose of 10 mgm. per kgm. This confirms the work of Hammet and Nowrey (4) who found that a single dose of arsenic trioxide administered subcutaneously at this level invariably killed normal animals in the 120 to 150 day age group.

It was also observed that arsenic poisoning is accompanied by a hypothermia evident even in cases of acute poisoning in which the animal dies in 15 minutes after injection. Many other drugs, including the cinchona bases, exhibit the same effect and Bodansky and Dawson (5) have utilized it in relation to toxicity determinations.

Hypothermia is exhibited by normal rats after injections of arsenic trioxide at as low a level as 4 mgm. per kgm. Twenty-one days after being placed on the 10 mgm. per kgm. level the rats of group 1 showed no drop in body temperature during the seven-hour period following injection, while two normal females given the same dose under the same conditions showed temperature drops of  $5^{\circ}\text{C}$ . and  $7.5^{\circ}\text{C}$ ., in the same period, dying during the night. The average rectal temperatures for two adapted rats and the two control animals are shown plotted against time in figure 1. All temperatures were obtained by inserting a thermometer in the rectum to a depth of three inches and reading the temperature after one minute.

The appearance of tolerance in the animals of group 1 led to further investigation of the phenomenon. Adaptation to intraperitoneally injected sodium arsenite was determined by taking the body temperature at intervals after injection of a non-lethal dose, until it began to return to normal. This procedure was followed until the temperature failed to drop after injection, at which time the animals were assumed to be adapted to that dose. They were then subjected to a higher dose and the degree of hypothermia compared with that produced in normal rats under the same conditions.

The results of a representative group of rats is shown in figure 2, which gives the average of values obtained on four three-month-old females (group 3). As it was found that marked fluctuations in room temperature during an experiment influence the body temperature response of the animals, the room temperature was maintained between  $24^{\circ}$  and  $26^{\circ}\text{C}$ . The course of adaptation to a dose of 6 mgm. per kgm. was followed by observing the body temperature for several hours after injection on the first, sixth, eleventh and eighteenth days. Adaptation was complete on the eighteenth day, a drop of only  $0.6^{\circ}\text{C}$ . being noted. This is hardly significant since the control animals given no arsenic trioxide showed a slight daily fluctuation during the one to two hour period following the taking of the first temperature reading in the morning.

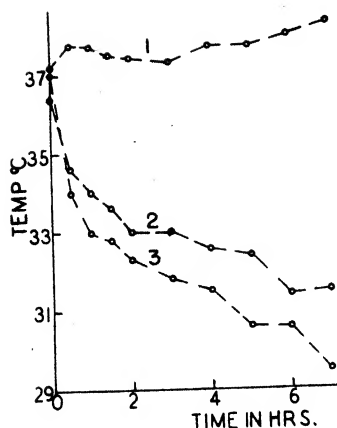


Fig. 1

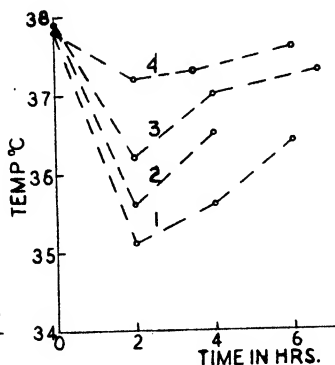


Fig. 2

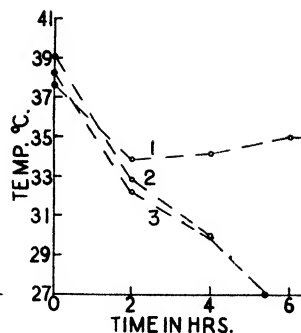


Fig. 3

Fig. 1. Post-injection time-temperature curves of normal rats and adapted rats of group 1 after injection of ten milligrams of arsenic trioxide per kilogram body weight.

1. Average temperatures of two adapted females. 2. Temperatures of one normal female after the first injection of arsenic trioxide. 3. Temperatures of one normal female after the first injection of arsenic trioxide.

Fig. 2. Post-injection time-temperature curves for rats of group 3, during an eighteen day series of injections of six milligrams of arsenic trioxide per kilogram body weight.

1. Average temperatures of four females after the first injection of arsenic trioxide. 2. Same as 1 after the sixth injection. 3. Same as 1 after the eleventh injection. 4. Same as 1 after the eighteenth injection.

Fig. 3. Post-injection time-temperature curves of normal rats and adapted rats of group 2 after the injection of 12 milligrams of arsenic trioxide per kilogram body weight.

1. Average temperatures of three adapted females. 2. Temperatures of one normal female; terminates at the death of the animal. 3. Temperatures of one normal female; terminates at the death of the animal.

TABLE 1

*Recovery of intraperitoneally injected arsenic trioxide*

RAT	TIME BETWEEN INJECTION AND WASHING	As <sub>2</sub> O <sub>3</sub> INJECTED	As <sub>2</sub> O <sub>3</sub> RECOVERED
		<i>gamma</i>	<i>gamma</i>
A—normal	30 minutes	2,000	40
B—normal	30 minutes	2,000	70
2	30 minutes	1,500	420
9	30 minutes	2,000	155
10	40 minutes	1,800	380
3	24 hours	835	340
6	24 hours	1,800	360
21	24 hours	1,830	380

The dose given the rats of group 3 was then raised to 10 mgm. per kgm. and held at this level for three days without any toxic symptoms. Three of the animals were then sacrificed for absorption studies reported below. The remain-

ing animal was given 12 mgm. per kgm. along with three normals, and the body temperatures noted for five hours after injection. All three normal animals died within five hours with temperatures four to five degrees below normal. The body temperature of the adapted rat fell 2°C. during the first hour after injection and then rose steadily to normal with no toxic symptoms.

Figure 3 gives the average temperature-time curve of three rats (group 2) given their first dose of 12 mgm. per kgm. after being on 8 mgm. per kgm. for ten days, and 5 mgm. per kgm. for five weeks, compared with two normal rats given their first dose of 12 mgm. per kgm. The normal rats died in four and six hours, respectively, evidencing marked hypothermia. The adapted animals showed some hypothermia but recovered.

In order to check the possibility that the tolerance observed might have been due to slow absorption through the membranes of the body cavity the following experiment was performed.

Six adapted and two normal rats were injected with known amounts of arsenic trioxide as indicated in table 1. At 30 minutes or 24 hours after injection as indicated in the table the animals were anesthetized with nembutal; the bellies shaved and opened along the midline. The peritoneal cavity was then washed out into a beaker with about 150 ml. of distilled water from a wash bottle. Care was taken to wash thoroughly, the organs being pulled outside the body and held apart with forceps during the washing. The amount of arsenic trioxide recovered was determined by the Gutzeit method (6). The residue of arsenic recovered from the adapted animals after thirty minutes is somewhat larger than that from normal rats; however the amount recovered after thirty minutes from adapted animals is of the same order of magnitude as that recovered after twenty-four hours, which probably represents the degree of saturation or the equilibrium within the body of animals receiving daily injections of arsenic trioxide.

#### SUMMARY

1. Rats have been shown to acquire a true systemic tolerance to arsenic trioxide in solution, injected intraperitoneally as sodium arsenite.
2. The toxicity of a dosage of arsenic trioxide and the progress of adaptation has been shown by following the hypothermia after injection.
3. The tolerance to injected arsenic trioxide was shown not to be due to decreased rate of absorption from the body cavity.

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# IN VITRO AND IN VIVO STUDIES OF THE EFFECT OF ARSENITE ON THE RESPIRATION OF RAT TISSUES

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The symptoms of both acute and chronic arsenic poisoning are given in works on pharmacology, as well as the specific injury to various tissues. Rats given 25 mgm. of arsenic trioxide, as a solution of sodium arsenite, per kilogram body weight may die in 15 minutes with the characteristic symptoms. Animals on lower but still lethal doses may develop the same syndrome in the course of a twenty-four hour period. Rats may be adapted to arsenic trioxide by the injection of sublethal doses over a long period of time (1). While normal rats show a marked hypothermia when injected with solutions of sodium arsenite, adapted rats show much less or no drop in body temperature.

The hypothermia follows immediately after the injection of arsenic and may be due either to a direct effect on the temperature regulating center in the hypothalamus, to some action on the vasodilator centers, or to a lowering of the basal metabolic rate which might conceivably be due to reduction of the respiratory rate of the individual body tissues.

With the object of determining the cause of the hypothermia, studies were made of the effects of arsenic trioxide on cerebral cortex, diaphragm, kidney cortex, and liver. In this work the Warburg technique (2), standard for determining the effects of drugs on tissue respiration, was used.

It has been known for some time that solutions of arsenic trioxide will reduce tissue respiration in vitro (3) and some action-concentration curves are available (4, 5), but it was deemed advisable to prepare complete action-concentration curves for the tissues used in this study in order to check in vivo with in vitro effects. The data for cerebral cortex, diaphragm, kidney cortex and liver are illustrated in figures 1 to 4 inclusive, in which percentile reduction is plotted against log concentration of arsenic trioxide. The points on the action-concentration curves were determined by adding dissolved arsenic trioxide (sodium arsenite) in suitable concentration to normal tissue slices whose respiration rates had been followed during the 40 to 60 minutes previous to the addition of the arsenic trioxide solution. In some cases the respiration rates did not fall to a constant level immediately and, therefore, instead of comparing normal respiration rates with those at a specified time after addition to determine the effect, areas under the time-oxygen consumed per hour per gram curves were compared before and after adding the arsenic trioxide. All points were figured on the basis of 40 to 60 minute periods before addition, and 40 to 70 minute periods after addition. Each point stands for the percentile reduction of a single slice of tissue by the indicated concentration of arsenic trioxide.

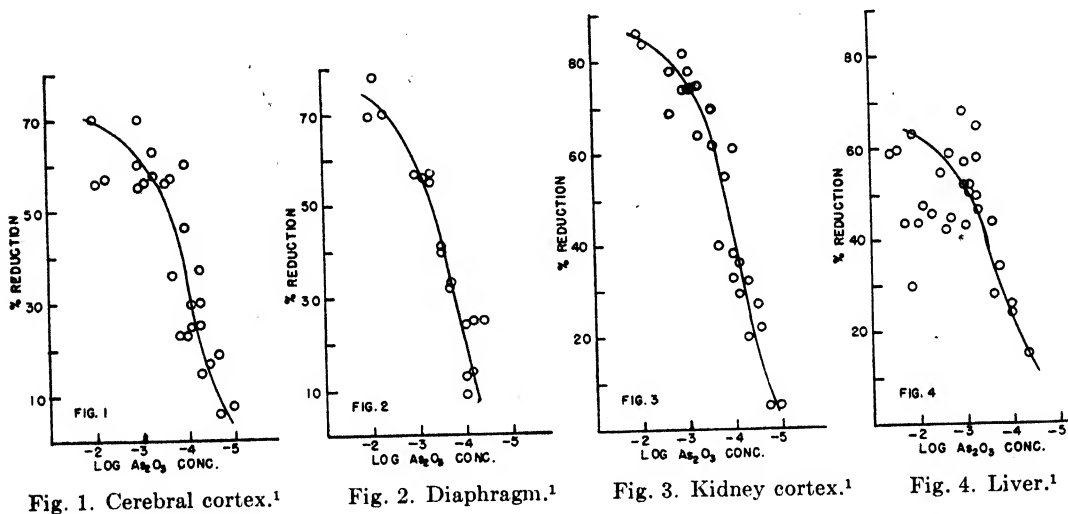
Since the action-concentration curves indicated that reduction of even the



most sensitive tissues was not to be expected on a minimum lethal dose (10 mgm./kgm.), it was decided to use 25 mgm. per kgm. as the dose for determining *in vivo* effects of arsenic trioxide. The method of procedure is described below.

The experimental animal was injected intraperitoneally or subcutaneously with 25 mgm. per kgm. of arsenic trioxide and sacrificed when anoxia was indicated by gasping respiration. In those animals receiving intraperitoneal injections, this stage was reached in 15 to 20 minutes, whereas those injected subcutaneously did not show the symptoms for from 40 to 60 minutes.

Tissues were removed immediately after sacrifice, prepared for respiration studies in a cold moist chamber (15°C.) and placed in a 38°C. bath within 30 minutes. The medium consisted of 0.9 ml. of Kreb's calcium free Ringer's solution. Liver and kidney slices were prepared with a Martin tissue slicer.



Brain slices were cut with the aid of a razor and template (6) and diaphragm was snipped with scissors into pieces of suitable size.

The *in vivo* effects of arsenic trioxide are summarized in table 1. The  $QO_2$ 's of the tissues from the poisoned animals are compared with the  $QO_2$ 's of normal tissues subjected to the same treatment. Since diaphragm, kidney and liver are in or adjacent to the peritoneal cavity, comparisons were made of the effect of arsenic trioxide injected intraperitoneally and subcutaneously. All  $QO_2$ 's are calculated 30 minutes after the first setting of the manometers.

**DISCUSSION.** The hypothermia is probably due to heat losses caused by vasodilatation, augmented by the lowered basal metabolic rate evident after the administration of arsenic trioxide. Vasodilatation might be the result of direct effects of arsenic trioxide on the temperature regulating or vasodilator centers in the central nervous system. Or, since arsenic has been called a capil-

<sup>1</sup> *In vitro* reduction of tissue respiration by arsenic trioxide.

lary poison, the vasodilatation might be due to a direct effect on the capillaries themselves. In the former case the effects might appear as an alteration of the respiratory rate of brain tissue. Unfortunately the latter case does not lend itself to study by the manometric technique.

The lowered basal metabolic rate should manifest itself by a reduction of the respiration rate of the individual tissues.

The in vitro studies, summarized on the action-concentration curves, indicate that if uniform distribution is assumed, reduced metabolism of liver and diaphragm is not to be expected, even on a dose of 25 mgm. per kgm. This dose should, however, cause reduction of brain and kidney tissue. Because in vitro effects on two out of four tissues were apparent at this dose, all in vivo work was done at this dosage level.

Manometric studies of the in vivo effects yielded results at variance with the theory of uniform distribution. Kidney cortex showed the expected reduction

TABLE 1

*Respiration rates of tissues of rats poisoned with arsenic trioxide compared with rates of normal tissues*

CEREBRAL CORTEX		DIAPHRAGM			KIDNEY CORTEX			LIVER		
B	C	A	B	C	A	B	C	A	B	C
QO <sub>2</sub> 's 2.50 <sub>3</sub>	2.13 <sub>6</sub>	1.40 <sub>4</sub>	0.95 <sub>3</sub>	1.50 <sub>6</sub>	3.52 <sub>2</sub>	3.07 <sub>2</sub>	3.00 <sub>6</sub>	2.25 <sub>3</sub>	1.50 <sub>4</sub>	1.78 <sub>6</sub>
2.40 <sub>3</sub>	2.47 <sub>6</sub>	1.40 <sub>4</sub>	0.90 <sub>2</sub>	1.57 <sub>6</sub>	3.36 <sub>4</sub>	3.85 <sub>3</sub>	4.75 <sub>6</sub>	2.20 <sub>3</sub>	1.44 <sub>2</sub>	1.90 <sub>6</sub>
2.33 <sub>3</sub>	2.34 <sub>4</sub>	1.38 <sub>3</sub>	0.90 <sub>4</sub>	1.84 <sub>6</sub>	3.68 <sub>2</sub>	3.96 <sub>4</sub>	4.90 <sub>6</sub>	2.32 <sub>4</sub>	0.90 <sub>3</sub>	1.78 <sub>6</sub>
		1.22 <sub>4</sub>				2.20 <sub>2</sub>	4.72 <sub>2</sub>	2.13 <sub>2</sub>		1.90 <sub>2</sub>

A—Subcutaneous injection.

B—Intraperitoneal injection.

C—Normal tissue.

Subscript number indicates number of slices averaged. Each value represents one animal.

but cerebral cortex continued to respire at a normal rate. Liver respiration was reduced when the arsenic trioxide was injected into the peritoneum, but was stimulated when the drug was injected subcutaneously. Diaphragm respiration was reduced under both conditions of injection, but more strongly in the case of intraperitoneal administration.

The results of the in vivo work lead to a number of interesting conclusions. The absence of any effect on the respiration of cerebral cortex does not favor the hypothesis of a direct action of arsenic trioxide on centers of the central nervous system. If arsenic trioxide has an effect on nerve tissue, it is not apparent in respiration studies.

The stimulating effect on liver respiration when the arsenic trioxide is administered subcutaneously may be related to a detoxification mechanism in that organ. The apparently anomalous reduction of liver respiration when the drug is given intraperitoneally is probably due to absorption of the drug by the intestinal capillaries which eventually carry the blood to the liver through the

portal vein. The liver is probably flooded with arsenic trioxide in amounts too great for it to handle. The resulting cellular damage appears as reduced respiration.

The reduction of diaphragm respiration indicates that the hypothermia may in part at least be due to a reduction in the heat production of the muscle tissues, which make up about 40 per cent of the body weight of the rat (7) and about 33 per cent of the heat production (8). The higher degree of reduction in the case of intraperitoneal injection is probably due to the exposure of the muscle to a high concentration of the drug immediately after injection and, consequently, it suffers greater damage.

Reduction of kidney respiration might be expected because these organs help excrete arsenic trioxide and a high concentration of the drug may rapidly be built up in them. As shown by the action-concentration curve for the organ, kidney is very sensitive.

The four tissues on which in vitro and in vivo effects were determined were chosen because it was felt that effects on them would be significant. Arsenic trioxide might directly affect the central nervous system, represented by cerebral cortex. Effects on diaphragm would indicate changes in the muscle system. Liver is concerned with detoxification and elimination (9) and kidney with elimination. Although other tissues undoubtedly would exhibit effects capable of interpretation by the Warburg technique, those chosen are sufficiently representative to indicate certain systemic actions of arsenic trioxide.

#### SUMMARY

1. By tissue respiration studies, arsenic trioxide action-concentration curves have been prepared for cerebral cortex, diaphragm, kidney cortex and liver in the albino rat.

2. Fatal doses of arsenic trioxide administered subcutaneously in vivo have no effect on cerebral cortex respiration, cause reduction of diaphragm and kidney respiration, and induce stimulation of liver respiration.

3. Fatal doses of arsenic trioxide administered intraperitoneally in vivo have no effect on cerebral cortex respiration, and cause reduction of diaphragm, kidney cortex and liver respiration.

4. The absence of effect on the cerebral cortex respiration, while the respiration of the muscle tissue of the diaphragm is reduced, would suggest that the hypothermia of arsenic poisoning is influenced more by a decreased rate of energy metabolism of the individual tissues than by a direct effect on the temperature regulating center in the central nervous system.

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# THE NATURE AND MECHANISM OF SHOCK PRODUCED BY THE INTRAVENOUS INJECTION OF CHYMOTRYPSIN<sup>1</sup>

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Chymotrypsin is a proteinase (1) that has been isolated by Kunitz and Northrop from the pancreas of cattle (2). It can be obtained in the form of a crystalline protein. The proteolytic action of chymotrypsin is very similar to that of trypsin although, as shown by Kunitz and Northrop, the hydrolysis of casein by the two enzymes occurs at different peptide linkages (2). Trypsin clots blood but, under ordinary conditions, does not clot milk. However, chymotrypsin clots milk but not blood (2). The optimum pH for the activity of chymotrypsin is between 7 and 9 (2), so that it is actively proteolytic at the normal pH of the blood.

The physiologic effects of intravenously injected proteolytic enzymes are not at present completely understood. Much work has been done on the effects of the injection of trypsin on physiological reactions in animals (3-4). According to Dragstedt and Rocha E Silva, trypsin produces a type of shock that is very similar to anaphylactic and peptone shock (3). A study of the action of trypsin given intravenously may lead to a better understanding of the mechanism of anaphylactic and peptone shock, and perhaps of other types of shock where proteolytic enzymes may play a rôle (5). However, in the case of trypsin, the interpretation of the results is obscured by the fact that trypsin brings about intravascular coagulation when it is injected intravenously. Therefore, it is difficult to evaluate in a given experiment what changes result from the clotting activity and what changes result from the proteolytic activity of trypsin, since clotting agents such as thrombin, and proteolytic agents such as trypsin both produce shock in animals (4).

Chymotrypsin does not clot blood and, therefore, is particularly suitable for investigating the physiologic effects of proteolytic enzymes because it is available in pure form and has an optimum pH in the neighborhood of the pH of the blood. The present report describes some of the changes that follow the intravenous injection of chymotrypsin into dogs.

**MATERIAL AND METHODS.** Twenty-eight mongrel dogs ranging in weight from 6 to 14 kgm. were used as experimental animals. They were anesthetized by the intravenous injection of 0.4 ml. per kgm. of a 10 per cent aqueous solution of sodium pentobarbital, except when otherwise indicated. Additional small quan-

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<sup>2</sup> Died on January 3, 1945.

tities of sodium pentobarbital were given during the course of the experiment when necessary to maintain the anesthesia.

Blood pressure recordings were made by a mercury manometer connected with either a needle or a cannula inserted into the femoral artery.

Intravenous injections were made into one of the jugular veins, and blood samples were obtained from another vein by syringe and needle.

The anticoagulant used for hematocrit determinations was a mixture of ammonium and potassium oxalates. For all other studies 1 ml. of a 2.5 per cent solution of sodium citrate per 9 ml. of blood was used. Plasma was obtained by centrifuging blood at 2500 r.p.m. for 15 minutes.

The chymotrypsin was a crystalline preparation made according to the method of Kunitz and Northrop (2)<sup>3</sup>, and contained about 50 per cent of  $MgSO_4$ . For intravenous injections it was dissolved immediately before use into a volume of saline equal to 1 ml. per kgm. of body weight.

Determinations of the blood coagulation time, the fibrinogen concentration and prothrombin time of the plasma were carried out as described elsewhere (4). When prothrombin time was determined on plasma containing an abnormally small quantity of fibrinogen, fibrinogen was added to the plasma before the determination.

Non-protein nitrogen of the plasma was measured by the usual micro Kjeldahl method.

Hematocrit readings were made by the technique of Wintrobe (6).

**RESULTS.** 1. *Lethal dose.* Five dogs received doses varying from 3 to 7 mgm. of chymotrypsin per kgm. of body weight and all survived; 18 dogs survived after receiving from 25 to 35 mgm. per kgm.

Three dogs received 40 mgm. per kgm. and died within 4 hours, yet 2 dogs received 50 mgm. per kgm. and survived. There was one dog which received 60 mgm. per kgm. and died.

2. *Digestion of plasma proteins by chymotrypsin.* To study the action of the chymotrypsin on the proteins of the plasma, 20 mgm. of chymotrypsin were added to 10 ml. of citrated dog plasma at pH 7.4. The mixture was incubated at 37°C. and samples were taken at intervals for determination of non-protein nitrogen, total protein and prothrombin time. A simple qualitative test for the detection of a decrease in the concentration of the fibrinogen in plasma consisted of the addition of thrombin to the mixture. The absence of coagulation indicates a marked decrease in concentration of fibrinogen. The results of one experiment (table 1) show that digestion as measured by increase in non-protein nitrogen took place to a slight extent and ceased after the first four hours. The fibrinogen and prothrombin of the plasma were decreased after two hours of incubation with chymotrypsin. This experiment was repeated several times with similar results.

3. *Effect on blood coagulation mechanism.* a. *In vitro.* Chymotrypsin had no accelerating effect on the coagulation of normal blood *in vitro*. Large quantities delayed the clotting time, as the fibrinogen and prothrombin underwent proteolysis (table 1).

<sup>3</sup> Obtained from the Plaut Research Laboratory, Bloomfield, N. J.

A typical experiment is shown in table 2. While trypsin did shorten the coagulation time of normal blood, chymotrypsin did not. Similarly, chymotrypsin did not shorten the clotting time of hemophilic blood *in vitro*.

b. *In vivo*. Table 3 shows the effect of the intravenous injection of varying doses of chymotrypsin on the clotting time of dog's blood. A prolongation of the

TABLE 1

*Effect of chymotrypsin on prothrombin, fibrinogen, total proteins and non-protein nitrogen of citrated plasma from dog's blood (in vitro)*

TIME	A. 10 ML. OF PLASMA + 20 MG. CHYMOTRYPSIN— 37°C., pH 7.5				B. CONTROL: 10 ML. OF PLASMA AT pH 7.5 INCUBATED AT 37°C.			
	Prothrom- bin time	Fibrinogen*	Total Proteins	N.P.N.	Prothrom- bin time	Fibrinogen*	Total Proteins	N.P.N.
	seconds		gm. per 100 ml.	mgm. per 100 ml.	seconds		gm. per 100 ml.	mgm. per 100 ml.
0 (control).....	11	Present	5.40	21	11	Present	5.38	22
2 hours.....	33	Absent	5.28	28	15	Present		23
4 hours.....	+60	Absent	5.26	35	15	Present		20
24 hours.....	+60	Absent	5.29	35	29	Present	5.40	23

\* Presence or absence of fibrinogen detected by the addition of thrombin.

TABLE 2

*Comparison between the effect of trypsin and chymotrypsin on the coagulation time of normal human blood at 37°C. Effect of chymotrypsin on hemophilic blood*

TUBE NO.	CHYMO- TRYPSIN	NORMAL BLOOD	CLOTTING TIME	TUBE NO.	TRYPSIN	NORMAL BLOOD	CLOTTING TIME	TUBE NO.	CHYMO- TRYPSIN	HEMO- PHILIC BLOOD	CLOTTING TIME
	mgm.	ml.	min.		mgm.	ml.	min.		mgm.	ml.	min.
1	0	2	15	1	0	2	8	1	0	2	65
2	0	2	16	2	0	2	9	2	0	2	70
3	0.05	2	15	3	0.05	2	8	3	0.05	2	65
4	0.05	2	15	4	0.05	2	8	4	0.05	2	70
5	0.1	2	16	5	0.1	2	2½	5	0.1	2	68
6	0.1	2	17	6	0.1	2	2½	6	0.1	2	75
7	0.25	2	30	7	0.25	2	2	7	0.25	2	+80
8	0.25	2	33	8	0.25	2	2	8	0.25	2	+80
9	0.5	2	+60	9	0.5	2	5	9	0.5	2	+80
10	0.5	2	+60	10	0.5	2	5	10	0.5	2	+80
11	1.0	2	+60	11	1.0	2	+60	11	1.0	2	+80
12	1.0	2	+60	12	1.0	2	+60	12	1.0	2	+80

clotting time was observed 10 minutes after the injection of 20 to 40 mgm. per kgm. of chymotrypsin into 3 dogs (nos. 6, 7, 4). The prolongation of the clotting time lasted for from 20 to 40 minutes after the injection. Doses of 4 mgm. per kgm. and less did not affect the clotting time in 6 dogs.

4. *Persistence of action of chymotrypsin in vivo*. The proteolytic activity and persistence of effect *in vivo* of intravenously injected chymotrypsin was investigated on 2 dogs. A typical protocol on one dog follows: 27.5 mgm. per kgm of

chymotrypsin were injected intravenously and the clotting time, fibrinogen level and prothrombin time were measured at intervals. The results (table 4) show that the prolongation of the clotting time following the injection of chymotrypsin

TABLE 3

*Effect of intravenous injections of chymotrypsin on the coagulation time in dogs*

DOG NO.	CHYMO- TRYPSIN  mgm. per kgm.	CLOTTING TIME* (MINUTES, ROOM TEMPERATURE)							
		1	2	3	4	5	6	7	8
1	3	15	16	14			15		
2	3.7	23	17	18	15				
3	10	15	14	13	18				
4	5	18	18	17	14				
5	4.5	18	17	16	17	14	15	15	
2	6.7	15	19				15		
6	40	12		30	+90		12		
7	30	9	45		90			20	8
4	20	11	29		40			15	

\* 1 = before injection

2 = 1-10 minutes after injection

3 = 10-20 minutes after injection

4 = 20-40 minutes after injection

5 = 60-90 minutes after injection

6 = 90-120 minutes after injection

7 = 120-240 minutes after injection

8 = 240-360 minutes after injection

TABLE 4

*Effect of intravenous injection of 27.5 mgm. of chymotrypsin per kgm. on clotting time, prothrombin time and plasma fibrinogen\**

	TIME FROM INJECTION	PROTHROMBIN TIME	PLASMA FIBRINOGEN	CLOTTING TIME AT ROOM TEMPERATURE
		seconds	mgm. per 100 ml.	minutes
1	Before	15	259	9
2	5 minutes	39	0	45
3	37 minutes	32	187	90
4	157 minutes	21	213	20
5	335 minutes	21	187	8
6	24 hours	17	203	10
7	48 hours			11

\* Dog 7, anesthetized.

was accompanied by marked prolongation of the prothrombin time and diminution of the fibrinogen of the plasma. Blood sample 2 in table 4 paradoxically clotted in 45 minutes, although the chemical determination of the plasma fibrinogen of the same sample of blood, which was carried out later, showed that no fibrino-



gen was present. It was probable, therefore, that all or some of the samples of citrated plasma contained some chymotrypsin and if such were the case, the results of the fibrinogen and prothrombin determination would represent the combined proteolytic activity *in vivo* and *in vitro* after the blood sample had been taken and before the determinations were carried out. Since there is no known specific inhibitor of chymotrypsin and the non-specific inhibitors, such as heat or pH changes, also interfere with the determination of fibrinogen and prothrombin, the following experiment was carried out in an attempt to determine how much of the digestive process was actually taking place *in vivo* and for how long after the injection chymotrypsin persisted in the blood. Citrated blood samples were

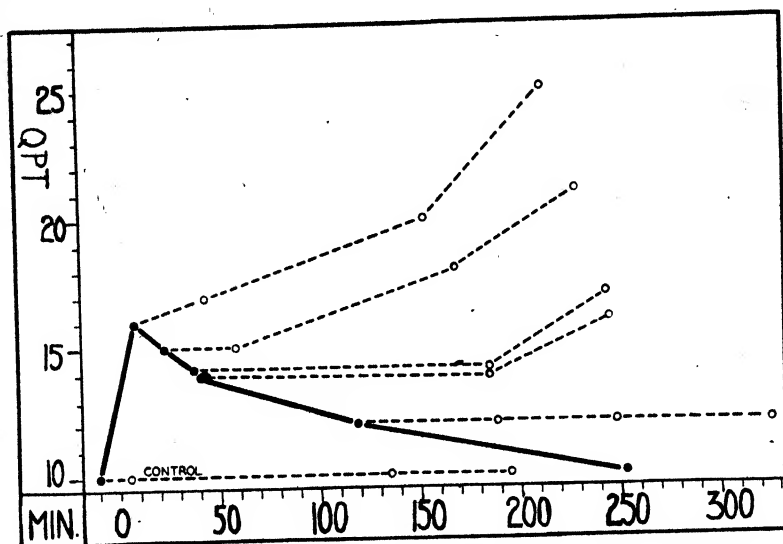


Fig. 1. Persistence of action of chymotrypsin *in vivo*. Dog 6 received 27.5 mgm. per gm. of chymotrypsin intravenously. Quick prothrombin time was determined immediately (solid line) and, on each sample, after incubation at 37°C. (open circles). Quick prothrombin time (QPT) is expressed in seconds.

taken at intervals from a dog which had received an injection of 30 mgm. per kgm. of chymotrypsin. The tubes containing the samples of blood were immersed immediately in cracked ice, centrifuged in melting ice, and then determinations of the prothrombin time and non-protein nitrogen were carried out. After the first prothrombin and non-protein nitrogen determinations, each sample was kept at room temperature and the prothrombin time and non-protein nitrogen level determined at intervals and compared with the initial values (figs. 1 and 2).

Fibrinogen determinations could not be used in this experiment as an index of protein digestion because complete fibrin formation requires one hour at room temperature, and this length of time is sufficient to allow digestion to proceed. The results of the prothrombin time determinations in this experiment are shown in figure 1, and the results of the non protein nitrogen determinations in figure 2.

Proteolytic activity was present in the circulating blood as long as 40 minutes after the injection (figs. 1 and 2) but was absent after 2 hours. (figs. 1 and 2).

5. *Effect on blood pressure.* The immediate effect on the blood pressure of various doses of chymotrypsin injected intravenously is shown in figure 3. There is an immediate depressor effect, the intensity of which was roughly proportional to the amount of chymotrypsin injected. With amounts of from 4 mgm. to 25 mgm. per kgm. the immediate depressor effect increased progressively. Three mgm. per kgm. caused no demonstrable effect on the blood pressure. This immediate depressor effect was of a transient nature and was characterized by a rapid fall which was followed by a return to normal within a few minutes.

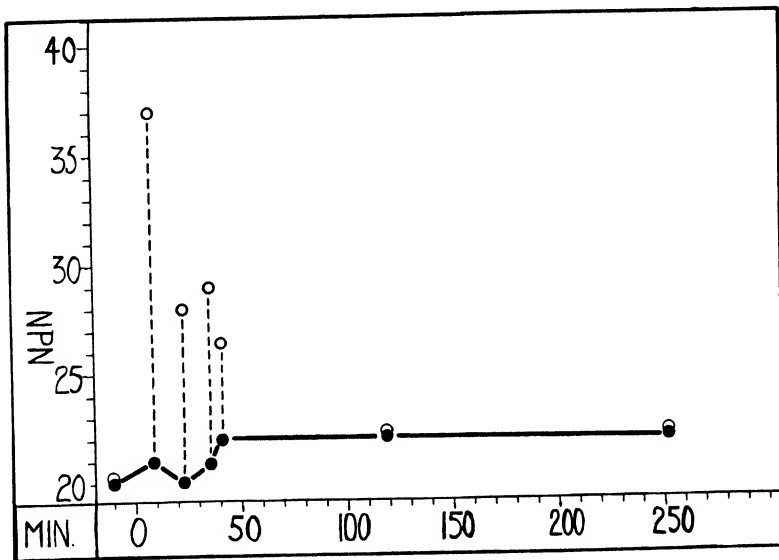


Fig. 2. Persistence of action of chymotrypsin *in vivo*. Dog 6 received 27.5 mgm. per kgm. of chymotrypsin intravenously. Non protein nitrogen determinations on the plasma were done immediately (solid circles) and on each sample of plasma after incubation at 37°C. for 24 hours (open circles). Non protein nitrogen (NPN) is expressed in milligrams per 100 cc. of plasma.

The blood pressure changes were followed over a long period of time in 10 dogs that received from 25 to 50 mgm. per kgm. of chymotrypsin. The results are shown in table 5. A pronounced fall in blood pressure during the first 90 minutes following the injection was observed in 4 dogs that were given 40 mgm. per kgm. (dogs 19, 20, 22, 25), and in 3 dogs that were given 45 to 50 mgm. per kgm. (dogs 23, 24, 26). The three animals that had received less than 40 mgm. per kgm. showed less marked depression of the blood pressure.

6. *Effect on the hematocrit.* Hematocrit measurements at intervals were made in 12 dogs that had received from 25 to 50 mgm. per kgm. of chymotrypsin intravenously. In 4 dogs receiving 25 mgm. per kgm., only small changes were observed (table 5, dogs 12, 13, 17, 18). All the others had received from 33 to

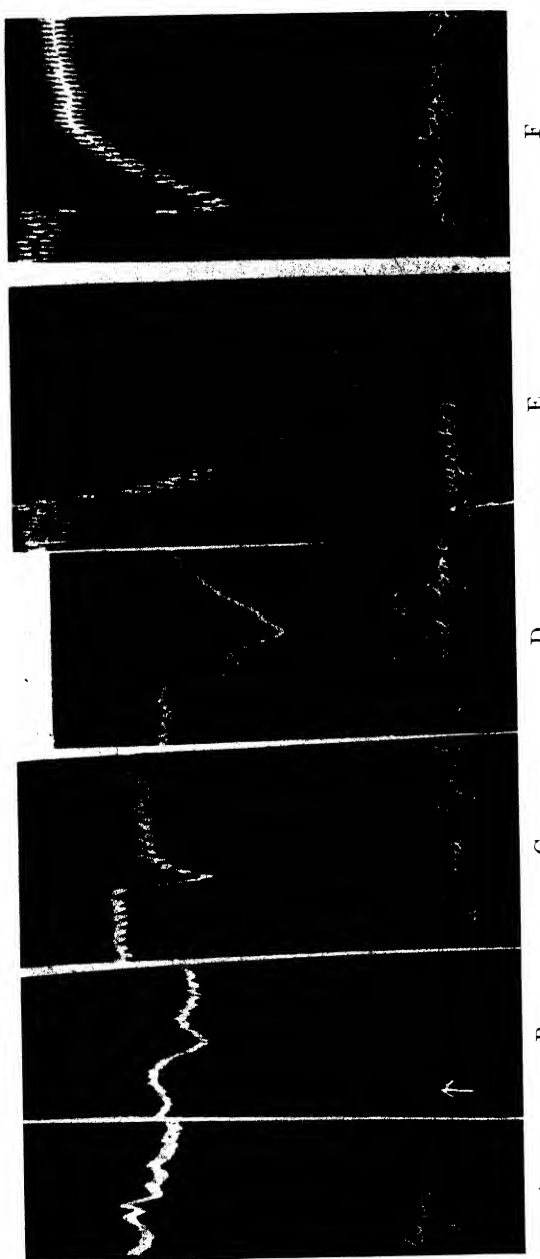


Fig. 3. Recording of blood pressure in dogs receiving intravenous injections of chymotrypsin (A,B,C,D,E.) and trypsin (F.).

- A: dog 1. 3 mgm. of chymotrypsin per kgm.
- B: dog 2. 4 mgm. of chymotrypsin per kgm.
- C: dog 4. 5 mgm. of chymotrypsin per kgm.
- D: dog 3. 10 mgm. of chymotrypsin per kgm.
- E: dog 26. 45 mgm. of chymotrypsin per kgm.
- F: dog 1. 2 mgm. of trypsin per kgm.

50 mgm. per kgm. of chymotrypsin, and they showed marked changes in the hematocrit values during the first 3 to 4 hours following the injection.

As a control experiment two dogs were anesthetized with sodium pentobarbital and determinations of the hematocrit values were made, at intervals for 4 hours.

TABLE 5

*Effect of intravenous injection of chymotrypsin on the hematocrit and blood pressure*

DOG NO.	NEMBUTAL	QUANTITY OF CHYMOTRYPSIN	HEMATOCRIT TIME FROM INJECTION (MINUTES)												
			0	30	60	90	120	150	180	210	240	270	300	330	500
	mgm. per kgm.	mgm. per kgm.													
12	40	25	44	44				48.7		50					
13	40	25	35.2	34.9		38		41.8			44				49.6
17†	25	25	35.4		34.5		33.4		33.6			33.3			45
18†	25	25	50		43		47.5		49.6			53.8			
19†	25	40	46		58										
20†	25	40	31				42			43					
21†	25	33	50				56				56				
22†	25	40	47					55			54				
23†	25	50	37	39	49				43		42				
24†	25	50	44	46					56		54				
25*	25	40	31	32	33		33		32		29				
26*	25	45	32										27		

DOG NO.	BLOOD PRESSURE (MM. Hg) TIME FROM INJECTION (MINUTES)													OUTCOME
	0	30	60	90	120	150	180	210	240	270	300	330	500	
12														Killed after 5½ hours
13														Killed after 5½ hours
17†	128		71		96		106			110				Killed after 4½ hours
18†	150		144		140		122			120				Killed after 4½ hours
19†	135		80											Died after 1½ hours
20†	120			76				40						Died after 3½ hours
21†	144				120				130					Killed after 4 hours
22†	150								140					Killed after 4 hours
23†	104	122	40	128			110							Killed after 4 hours
24†	140	30					76		94					Killed after 4 hours
25*	140	80	108	92	124	116					70	56		Killed after 5 hours
26*	160	52		68										Died after 1½ hours

\* Splenectomized.

† Post mortem examination made.

No chymotrypsin was injected into these animals and no increase in the hematocrit values were observed.

Contraction of the spleen can cause marked changes in the hematocrit values and red cell counts of the blood of dogs in a number of experimental conditions. Therefore, two dogs were splenectomized with sterile precautions and after they had recovered from the operation they were given intravenous injections of 40

to 45 mgm. per kgm. respectively of chymotrypsin. Although a marked and profound fall in blood pressure was produced in these two dogs, no rise in hematocrit was observed for the duration of the experiment (table 5, nos. 25, 26).

7. *Post-mortem examination.* Eight dogs that had received one single intravenous injection of chymotrypsin either died or were killed and post-mortem examinations were carried out immediately after death. The quantity of chymotrypsin given to each dog and the time at which post-mortem examination was done is indicated in table 5. Sections of tissues for histological examination were put into Zenker's solution within 15 minutes after the death of the animal. On gross appearance the organs uniformly showed congestion and some cyanosis. Sections for microscopical examination of the heart, pancreas, kidneys and adrenals were made. Morphologic changes of an acute nature were observed microscopically in the liver, the kidneys and the adrenals. In dogs 21, 22, 23, 24, there was central necrosis of the liver. In dog 20 there was central necrosis with acute passive congestion. In dog 17 mid-zone foci of polymorphonuclear cells suggested focal necrosis of liver but no actual necrotic cells could be seen. Dog 19 showed only passive congestion of the liver.

The adrenals of dogs 17 and 18 showed marked polymorphonuclear leucocytic infiltration of the zona fasciculata, more intense and diffuse in dog 18. This may indicate reaction to early necrosis or capillary damage. The kidney tubule epithelium of dogs 17, 22, 23 and 24 showed considerable granularity and loss of cell outline. The appearance was not different from that seen in marked post-mortem change. However, in view of the early Zenker's fixation of the tissues there is a possibility that the change seen represents antemortem tubule damage. Casts of packed epithelium were observed in the collecting tubules of dog 23. No abnormalities were found in the sections of the pancreas from dogs 19, 20, 21, 22, 23, 24 and in the hearts from dogs 21, 22, 23, 24. A typical picture of the microscopic aspect of the kidneys, adrenals and liver is shown in figure 4.

DISCUSSION. On the basis of the experimental data presented, some comparisons of the action of trypsin and chymotrypsin can be made. The first difference is to be found in the action on the coagulation of the blood. Trypsin clots oxalated plasma or blood and accelerates the clotting time of whole blood. In large quantities, however, trypsin delays the clotting time of whole blood due to rapid fibrinolysis (7). Chymotrypsin does not clot oxalated plasma and does not accelerate the coagulation time of whole blood. In large quantities it delays the clotting time of whole blood by causing proteolysis or some other modification of fibrinogen and prothrombin (tables 1 and 2).

Chymotrypsin was found in dogs to be lethal in doses of between 40 and 60 mgm. per kgm. Twenty-five milligrams per kgm. or less did not cause death in the experimental animals. At necropsy, no intravascular coagulation was found by macroscopic examination, as was to be expected from the fact that chymotrypsin does not cause blood to clot *in vitro*. In contrast with chymotrypsin, trypsin in rabbits and dogs was found to be lethal in doses of between 5 and 10 mgm. per kgm. (4). Post-mortem examination of animals injected with 2 mgm. per kgm. or more of trypsin frequently reveals intravascular blood clots, in agree-

ment with the fact that trypsin causes the blood to clot *in vitro* (7). After injection of trypsin into animals, the clotting time of the blood often is found to be markedly prolonged and this prolongation has been attributed to an intravascular coagulation accompanied by decreased fibrinogen and prothrombin concentrations in the circulating blood. Such defibrination of the circulating blood produced by the injection of trypsin can be prevented by the preliminary administration of heparin to the animal, suggesting that the defibrination is caused by the clotting activity of the trypsin (4).

Chymotrypsin prolongs the clotting time of dog's blood but only when 20 mgm. per kgm. or more are injected. The data reported here indicate that chymotrypsin may digest or completely modify the physiologic properties of the fibrinogen and prothrombin of the plasma *in vivo* and *in vitro*, and it is probable



Fig. 4A. Liver, dog 20, showing central necrosis with acute passive congestion. Methylene blue and phloxine.  $\times$  about 45

Fig. 4B. Kidney, dog 21, showing granularity of tubule epithelium, loss of cell outline, and actual loss of cells. Methylene blue and phloxine.  $\times$  about 375

Fig. 4C. Adrenal, dog 18, showing marked polymorphonuclear leucocyte infiltration of zona fasciculata. Methylene blue and phloxine.  $\times$  about 375

that the delayed clotting time is due to the decrease in fibrinogen and prothrombin of the plasma brought about by the proteolytic action of the chymotrypsin.

As far as the effect on the blood pressure is concerned, the intravenous injection of trypsin into dogs produced a marked and rapid fall in the blood pressure. The initial effect of chymotrypsin on the blood pressure was much less intense, as a comparison between the tracings obtained with each enzyme will show (4). Part of the difference can be explained probably by the fact that trypsin clots blood while chymotrypsin does not. The difference in the peptide linkages of proteins attacked by each enzyme may contribute to the difference in physiological actions. This is suggested by the findings of Rocha E Silva who showed that histamine is released when smooth muscles are perfused with a solution of trypsin (3). He suggested that trypsin liberates histamine from protein-

histamine complexes (3). It is possible that chymotrypsin does not liberate histamine from these complexes, or does not do so to the same extent as trypsin (8). Perfusion experiments using a solution of chymotrypsin should clarify this point.

In plasma, chymotrypsin causes proteolysis of the fibrinogen and the prothrombin *in vitro* as well as *in vivo*.

The activity of intravenously injected chymotrypsin on plasma proteins persists *in vivo* for at least 40 minutes, as shown in figures 1 and 2, which fact makes the chemical determinations of fibrinogen and prothrombin difficult if not impossible as an index of the digestion of these substances in the circulating blood.

The action of chymotrypsin on the blood pressure in the experiments reported here is characterized by a marked vaso-depressive action persistent for 1 to 4 hours. These changes occurred only with doses of chymotrypsin of 25 mgm. per kgm. or higher. These doses produced a variable degree of rise in the hematocrit which was marked when a sufficient quantity of chymotrypsin was used. There was some parallelism between the effect on the hematocrit and the effect on the blood pressure. It is noteworthy that in the 2 dogs where no significant changes in blood pressure followed the injection of 25 mgm. per kgm. of chymotrypsin, no significant change in the hematocrit was observed (table 5, dogs 17, 18).

However, no changes in the hematocrit values were observed following the injection of 40 and 45 mgm. respectively of chymotrypsin per kgm. of body weight in 2 splenectomized dogs, although deep shock was produced in each of them (table 5, dogs 25, 26). This latter finding strongly suggests that contraction of the spleen is responsible for the rise in hematocrit observed in normal dogs after injection of chymotrypsin.

The morphologic changes observed in dogs after intravenous injections of chymotrypsin were most marked in the liver, and less marked lesions were also found in the kidneys and adrenals. It should be noted that in all cases tissues for examination were excised and fixed immediately after death. These morphologic changes are not specific and no explanation is offered for the mechanism of their production. It is possible that more definite changes in the organs would have been observed if the dogs had been kept alive for a few days after the injection of chymotrypsin and then killed for examination of the morphologic changes.

There are at least five experimental conditions characterized, in dogs, by a marked depression of the blood pressure accompanied by prolongation of the clotting time of the blood. They are anaphylactic shock, peptone shock, trypsin shock, the shock produced by the intravenous injection of thrombin and the shock produced by the intravenous injection of chymotrypsin. Three different mechanisms account for the prolonged clotting time in these five types of shock. In anaphylactic and peptone shock, the prolonged clotting time is due to release of heparin (3); in shock produced by trypsin and thrombin it has been explained by an intravascular defibrination (4); in shock produced by chymotrypsin, the present data indicate that it may be caused by a direct proteolytic action of the enzyme on the fibrinogen and the prothrombin in the circulating blood.

The mechanism of chymotrypsin shock is not explained by the data presented here, but in view of the proteolytic action of chymotrypsin *in vivo*, it is suggested that the shocking action may be brought about by the products of proteolysis of the animal's own proteins, but the results of *in vitro* experiments indicate that the amount of such proteolytic products must be extremely small. The ability of proteolytic enzymes to produce a shock like condition receives added significance from the fact that liberation of such enzymes in the blood stream is sometimes observed in traumatic and hemorrhagic shock in humans and experimental animals (9).

#### SUMMARY

1. The effects of intravenous injections of chymotrypsin into dogs was studied.
2. Chymotrypsin brought about proteolysis of fibrinogen and prothrombin in the circulating blood, thereby prolonging the clotting time.
3. The lethal dose of chymotrypsin was found to be between 40 and 60 mgm. per kgm. of body weight.
4. A prolonged depression of the blood pressure was observed with intravenous injections of from 40 to 50 mgm. per kgm.
5. The immediate and transient effect on the blood pressure of from 3 to 25 mgm. of chymotrypsin per kgm. was much less pronounced than the effect of comparable amounts of trypsin.
6. Pathological changes in the liver, kidneys and adrenals were observed after intravenous injection of large doses of chymotrypsin.

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# TRANSIENT AND PERMANENT AFTER-EFFECTS OF EXPOSURE TO OXYGEN AT HIGH PRESSURE<sup>1</sup>

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It is well known that the exposure of animals to O<sub>2</sub> at high pressure (OHP) i.e., to either pure oxygen at pressures in excess of one atmosphere, or to gas mixtures at pressures such that the partial pressure of the contained O<sub>2</sub> tension is greater than one atmosphere, causes them to manifest reactions which have been referred to as those of O<sub>2</sub> poisoning. These reactions, the outstanding features of which are dyspnea, motor disturbances, convulsions, and death if the exposure is severe, become particularly acute and striking in O<sub>2</sub> pressure at from 3 to 4 atmospheres and above, although there is a wide individual variation both in the susceptibility to this toxic action and in the severity of the reactions.

Recovery from these effects in many cases appears to be rapid and may seemingly be complete, but examinations of the various reports and experimental data reveals sufficient evidence to render of dubious validity the designation of this recovery as an invariable occurrence, and seriously to question the belief that it is instant or that it is always complete as has been held by some investigators. Animals which have been exposed to OHP, and particularly those which have been convulsed thereby, not infrequently retain motor disturbances or stuporous states with or without any associated motor disturbance. This persistence of post-decompressional effects, slight as they may be in some cases, indicates the retention of some etiological factors which influence the C.N.S. for some time after the animal's return to normal environmental conditions; conceivably it might be a retention of some toxic substance, or the injury to tissue, or a combination of factors.

A study of these post-decompressional reactions should therefore, help to clarify some features of the toxic influence of OHP. But such study has several inherent difficulties; these post-decompressional manifestations of C.N.S. involvement are frequently transient, and attempts to augment and prolong them by increased severity of the exposure to OHP, usually result in the death of the animals, owing either to profound C.N.S. effects, or to lung damage or to both. However, because most animals possess a pronounced capacity for recovery from OHP, and because the post-decompressional effects do persist for some time, the intensity of those effects should be enhanced and their duration prolonged, thus rendering them more amenable to study, by the use of successive exposures so spaced as to produce additive effects without at the same time introducing serious complications of pulmonary damage or causing death of the animals.

<sup>1</sup>Preliminary report in Fed. Proc. 2: 2, 1943.

Such procedures were therefore carried out in experiments on healthy young adult albino rats. The exposures to O<sub>2</sub> (U. S. P.) at pressures of 65 pounds (gauge) were made in a pressure chamber supplied with ventilating devices and soda-lime cartridge for CO<sub>2</sub> absorption; as an additional precaution against the accumulation of even small amounts of CO<sub>2</sub> in the respired gas—a factor of paramount importance where OHP is concerned—a layer of soda-lime was spread in the chamber beneath the false floor supporting the animals. Before each exposure the chamber was washed free of air by a rapid flow of O<sub>2</sub> from the supply tank. The temperature was maintained at about 24°C. and throughout the exposures the animals were under the observation of the operator outside of the chamber.

The duration of exposure was determined by the individual response of the animals so that since the susceptibility varied widely in different individuals the duration of exposure was not uniform in all cases. Decompression was usually begun either with the onset of those symptoms which commonly herald the approach of a convulsive attack, such as twitching of facial or body musculature, or, since some animals go into coma and succumb without being convulsed, when the respiratory difficulty had become pronounced. For the most part, therefore, the exposures were relatively short, thus minimizing the occurrence of lung damage and fatalities. But in spite of these precautions, severe convulsive seizures were precipitated in some animals without the usual premonitory signs; in some other experiments in which attempts were deliberately made to induce convulsive attacks, the animals occasionally failed to show any reactions other than hyperpnea and lethargy even after exposures as long as 40 minutes. The usual range in the duration of exposure, however, was from 10 to 25 minutes with an average of about 15 minutes.

Decompression was carried out in stages and occupied a period at least equivalent to the duration of the exposure to the increased pressure; this eliminated the possibility of bubble formation and its attendant complications.

The spacing and the total number of exposures used varied widely in different experiments and, like the duration of each exposure, were determined by the individual response of the animals, the severity of the residual effects following several exposures, and the results desired. In the earlier experiments involving successive exposures the animals were usually subjected to 3 exposures per day, spaced at intervals of from 3 to 4 hours, following which they were given a rest until the next morning at which time another series of 3 exposures were given, depending upon the condition of the animals. Except for the time during which the animals were in the pressure chamber they breathed room air at atmospheric pressure.

**RESULTS.** Pronounced decompressional and post-decompressional reactions commonly occurred following single exposures to OHP but the intensity and duration of these reactions showed wide individual variations. Those animals which were most profoundly affected during their stay at the increased pressure usually showed the more pronounced post-decompressional reactions but in this there were many notable exceptions and animals which had shown nothing more

than respiratory difficulty while under the increased pressure, often were among those which succumbed shortly after their return to normal pressure.

The susceptibility of the animals to the convulsant action of OHP was increased by successive exposure so that the length of exposure necessary to induce the typical premonitory symptoms of the convulsive attack was commonly shortened by as much as, and in some cases more than, 25 per cent; furthermore the severity of the reactions was increased by successive exposures. This increase in susceptibility, however, did not always continue indefinitely; after some six or eight exposures it increased more slowly and in some instances remained without much further change, unless the program of exposure was altered.

The character of the decompressional and post-decompressional reactions which occurred following the exposure to OHP suggested their classification into two phases: *a*, the acute, and *b*, the chronic. Both phases were sometimes observed following single exposures to OHP, particularly if the exposure was severe, but usually the chronic phase was not induced by one exposure alone.

*The acute phase.* In the acute phase are included all those disturbances observed during the period of decompression, and in the post-decompression period immediately following the animals' return to atmospheric pressure. These, for the most part must be simply the continuation of those responses to OHP initiated during the animals' stay at the increased pressure; in other words they represent a lag in recovery. The manifestations of the acute phase varied widely both in intensity and type of reaction.

Among the acute reactions observed during decompression, the convulsive seizures were the most striking, and deserve special mention. They occurred very commonly just after decompression was begun; animals which had shown only very slight, or even no reactions while under pressure were frequently severely convulsed during the decompression. These decompression convulsions were grossly similar to the oxygen convulsions precipitated in a few animals before decompression was begun and they may, like some of the other reactions seen in the acute phase, represent a delayed recovery from the effects induced by the  $O_2$  while under pressure. On the other hand the frequent postponement of their onset until after decompression had been started provides good reason to suspect that decompression itself introduced some specific factor which precipitated the attacks. In any case the decompressional convulsions as observed in our experiments cannot be attributed to bubble formation.

The acute reactions were not confined to the duration of actual decompression but extended into the post-decompressional period for various lengths of time. The post-decompressional convulsive seizures, however, were seldom as severe as those seen either during the maintenance of the pressure or during the decompression, but tonic spasms with opisthotonus, in some cases precipitated by handling the animals, were not infrequently observed. A less severe and less extensive but continuous hypertonicity with faintly palpable muscle tremors was present in some animals; others were distinctly hypotonic. Some were hyperexcitable and leapt into the air on being touched; in others the reflex response to nocuous stimuli was absent or diminished. Striking states of catatonia in

which the animals could be moulded into bizarre attitudes were also observed; some animals were comatose. Many of those individuals able to walk, did so very unsteadily and presented evidence of marked disturbances in equilibrium. Very commonly only stuporous and lethargic states without any accompanying motor disability were present.

The acute reactions in the post-decompressional period following a single exposure to OHP was frequently transient so that unless the reaction to the OHP was particularly severe, recovery appeared to be complete within an hour following the animal's return to atmospheric pressure. An interval of 3 to 4 hours between successive exposures was therefore usually more than sufficient for recovery from the acute reaction following a single exposure, or the first few of a series of moderately severe exposures. But continuation of the program of successive exposures progressively increased the severity, and prolonged the duration of the acute post-decompressional reactions to a point where lengthening the inter-exposure interval, or stopping the exposures for as long as a day or more, was necessary to permit recovery from the acute effects.

In spite of the precautions taken to prevent the loss of animals, many succumbed—some after only one or a very few exposures to OHP. But few animals died after having survived the acute phase of the decompressional and post-decompressional periods. The mortality was higher at the beginning of each new series of exposures; this, no doubt, was due in part to the selective action of the OHP and in part to a better evaluation of an animal's susceptibility after observations made on it during its first few exposures. The occurrence of these fatalities emphasizes the difficulty of accurately predicting without previous trial just where the lethal effects of OHP begin in different individuals.

After an initial diminution or disappearance following each exposure the acute post-decompressional effects merged with chronic changes which, for the most part, developed gradually with successive exposures, showed little or no diminution between exposures and formed a background upon which acute effects of subsequent exposures could still be superimposed.

*The chronic phase.* The outstanding feature of the chronic effects was a motor dysfunction, usually seen first in the fore legs; the digits remained continuously flexed and in walking, the fore limbs, if employed, were used in pegleg fashion or as skids; not uncommonly locomotion was accomplished in the manner of a kangaroo. In more advanced chronic disturbances the forelimbs were completely incapacitated and the animal assumed a hunched-up position; in still more severely affected animals all four limbs were incapacitated so that they were unable to stand or to right themselves, necessitating special attention in feeding and bathing.

Some of these features are shown in the accompanying illustrations. Figure 1 shows the condition in an animal 16 months after the last exposure to OHP: this animal typical of others similarly treated, was unable to right itself or to stand without aid, but with some help to prevent its falling sideways, the spasticity of its hind legs and tail supported its weight and the stiffened back maintained the body in the upright position shown. Figure 2A shows a somewhat less se-

verely affected animal. The kangaroo-like stance mentioned above is well illustrated in a third animal in figure 2B.

Some improvement was noted in the less severely affected individuals over a period of weeks or months after the last exposure to OHP, and a few of these seemed to have either recovered almost completely or made compensatory adjustments within periods of from four to six months. The large majority, how-



Fig. 1. Permanent spastic paralysis in the fore and hind limbs 16 months after the last exposure to  $O_2$  at 65 pounds' pressure. The rigidity of the hind legs and tail support the body weight, but animal falls when side support is removed. Forelimbs and paws completely incapacitated. This animal was subjected to 14 exposures, each of about 21 minutes duration, over a period of 8 days. Decompression time after each exposure was 20 minutes.

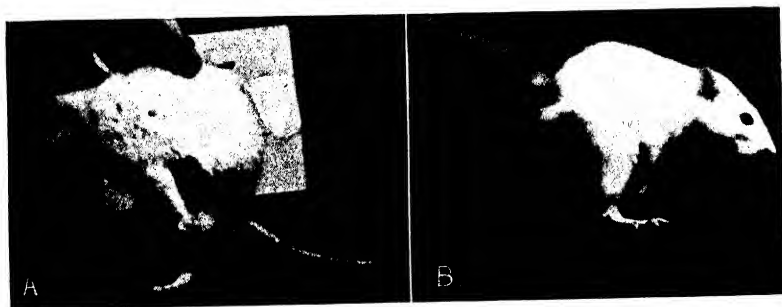


Fig. 2. A. Permanent spastic paralysis in fore and hind limbs following 10 exposures to OHBP, each of about 25 minutes' duration, over a period of 8 days. Decompression time after each exposure was 32 minutes.

B. Less severe paralysis in another animal. Note the kangaroo like stance which is assumed in many of these animals permanently affected.

ever, after showing some slight improvement, retained their disabilities permanently; at least at the time of writing these disabilities have been retained with no appreciable abatement for periods as long as 16 months. This chronic motor dysfunction is essentially a spastic motor paralysis: the rigidity of the involved limbs is very pronounced but under maintained force applied to the plantar surface of the hind feet it gives way temporarily in a clasp-knife manner.

The susceptibility of the animals to these chronic motor disturbances, like

that seen in the acute phase, varied widely in different individuals. In a few animals chronic changes were induced by a single exposure to OHP. Some of the more severe chronic effects were observed in those animals which suffered convulsive attacks, but such attacks are not essential to the development of this chronic motor dysfunction, since it occurred also in animals which had shown no obvious acute motor disability or convulsive seizure either during or after decompression.

The development of the chronic effects, like the augmentation of the acute effects, did not always progress indefinitely as the successive exposures were continued; some animals after having reached a certain stage of chronic involvement showed little further increase in disability even after prolonged extension of the initial program of exposures. But further augmentation of the chronic effects could be induced by increasing the duration of each exposure to the OHP or shortening the interval between successive exposures. In the less susceptible animals the interval between successive exposures was found to be an important determinant in the induction of the chronic phase; four exposures per day instead of the usual 3 distinctly hastened the onset and decreased the total number of exposures necessary to bring about chronic alterations.

In addition to these chronic motor paralyses some few animals gradually became lethargic and anorexic following successive exposure to OHP. These animals were peculiarly resistant to the convulsive action of OHP but a number succumbed after only a few exposures. Autopsies revealed the lungs in some, but not in all of these to be clear, so far as gross pathology was concerned.

**DISCUSSION.** One of the supposedly characteristic features of the reactions to OHP is the reversibility of the reactions when the animal is returned to normal atmospheric pressure; and it is true that some of the features of the response to OHP—especially the hyperpnea—is reversed by lowering the oxygen pressure, provided the exposure to the OHP has not been unduly prolonged and pulmonary damage has not been extensive (Bean, 1931). This reversibility of respiratory effects finds a ready and adequate explanation in the rôle which endogenous  $\text{CO}_2$  plays in poisoning by OHP.

The respiratory reactions resulting from the more severe exposures, however, are not always reversible even where lung damage is not entirely responsible for those reactions and it was suggested (Bean and Rottschäfer, 1938) that this lack of reversibility may be due to some irreversible alteration in nerve cells of the regulatory mechanisms of breathing as a result of the increased  $\text{CO}_2$  tension in the tissues, an increased acidity obtaining during the exposures, and a deleterious action of OHP itself. In any case, such results indicate that recovery does not invariably occur, even though the blood pressure be fairly well maintained.

Our present experimental results conclusively show that there are manifestations of C.N.S. involvement in poisoning by OHP other than those involving respiration and that these manifestations commonly persist for variable periods after the return of the animal to normal conditions and in some cases remain permanent. In single exposures, there must be sub-symptomatic effects in the C.N.S. which are retained for some time after apparent complete recovery.

The convulsive seizures observed by Bert (1878) in dogs exposed to hyperoxygenated air at increased pressures and which he attributed solely to oxygen poisoning, occurred, with few exceptions, either during or after decompression to normal atmospheric pressure. This persistence of effects after the removal of the original cause, led him to the belief that OHP produced some toxic chemical substance which outlasted the exposure to the OHP and which, acting on the C.N.S., primarily the spinal cord, gave rise to the convulsive seizures.

These experiments of Bert might seem to provide very early evidence that recovery from exposures to OHP is not "immediate," "instant" or "invariable" as some authors have reported (Shaw et al., 1934; Behnke et al., 1934). But an examination of Bert's reports reveals that the experimental procedures he employed do not justify his conclusion that the convulsions he described were simply those OHP. His decompressions were carried out entirely too rapidly even if he had employed pure  $O_2$  as the compression medium, let alone his use of hyperoxygenated air in which the  $O_2$  content was in some instances not more than 60 per cent. Gas emboli must then have contributed to, if they were not often the sole cause of, the precipitation of many of his decompression convulsions. Hill (1912) maintains that the convulsions Bert details as  $O_2$  convulsions were clearly the result of gas effervescence.

Bert's contention that the convulsions he described were caused by OHP alone, is open to question for another reason, viz., the high  $CO_2$  content of the respired gas. He felt convinced that the presence of  $CO_2$  (in some cases 8 to 10 per cent) was of no consequence under his experimental conditions in which his animals re-breathed compressed hyperoxygenated air. The post-decompressional reactions in Bert's animals must therefore have included not only the effects of gas effervescence, but also those of high  $CO_2$  concentrations, in addition to the toxic effects of OHP itself.

The report of Thompson (1889), whose experiments on OHP are open to less criticism on these grounds than are those of Bert, also shows, however, that animals which had been exposed to OHP did not recover immediately on their return to normal atmospheric pressure; additional evidence that the recovery from oxygen convulsions is not immediate is found in the work of Ozorio de Almeida (1934).

Furthermore in experiments in which the possibility of decompressional effervescence and  $CO_2$  effects have been eliminated by suitable precautions, one of us has observed over a period of years, that while the return to atmospheric pressure after relatively short exposures of unanesthetized rats is followed by what appears to be a rapid recovery, there often persists stupor or some minor motor dysfunction even though the reaction during the exposure to OHP may have been slight and the convulsive stage never reached. There is on the whole, then, considerable evidence that the recovery from exposure to OHP is by no means always immediate or instantaneous. Our observation that in most animals permanent disability is induced more readily by a program of 3 or 4 exposures per day than by 1 or 2 per day points to the possible importance of the over-lapping and the additive nature of the sub-symptomatic changes induced by each exposure.

Bert, having attributed the convulsive seizures and post-decompressional reactions to some intermediate toxic substance produced by the OHP, attempted to demonstrate the presence of such substance in the animals which had been convulsed, but neither his own nor somewhat similar attempts of subsequent investigators (Campbell, 1937; Bean and Bohr, 1940; Iwanow et al., 1934) were successful. Such negative results might be variously interpreted; they strongly suggest that no specific toxic substance is formed in exposures to OHP, yet it is conceivable also that a toxic substance thus far not demonstrated because of its transiency or its minute quantity might still induce tissue injury, which would then act as the immediate cause of the post-decompressional reactions. Earlier search for explanatory pathology in the C.N.S. (Bert, 1878; Shilling and Adams, 1933) has been unsuccessful.

In spite of the failures, up to now, to demonstrate either a toxic substance or tissue injury as the immediate cause of the convulsive seizure or post-decompressional reactions, the present experiments conclusively show that prolonged and permanent disabilities occasionally do occur following a single exposure to OHP, and that such permanent disabilities can be regularly developed by successive exposures. This must mean that in those cases where the acute post-decompressional effects of a single, or of the first few of a series of exposures are seemingly short lived, there still remain residual sub-symptomatic changes, which, with successive exposures, become additive and eventually result in a permanent objective symptomatology that cannot be explained as of "functional" origin (Smith, 1899). While apparent recovery from very severe reactions to OHP is frequently dramatic, the results of our present experiments are at distinct variance with the view that "complete recovery invariably follows removal from oxygen" (Behnke and Shaw, 1937).

The explanation for the acute and chronic reactions might then be somewhat as follows: The acute reactions which occur during the actual exposure of the animal to the OHP, those seen during the stages of decompression, and those of the immediate post-decompressional period, can be attributed to transient toxic substances together with an almost completely, but not immediately, reversible tissue injury which those substances induce. The accumulation of endogenous  $\text{CO}_2$  within the tissues due to a disturbed  $\text{CO}_2$  transport, the increased acidity and the direct toxic action of OHP on enzymes, all of which conditions obtain in OHP, serve as the transient toxic agents; neither the reactions nor the conditions demand the postulation of a specific intermediate toxic substance such as Bert suggested.

The chronic post-decompressional effects, manifest by persistent and permanent disability cannot however, be explained adequately on the basis of some toxic substance acting as an immediate cause. The more logical and well nigh inescapable interpretation would be that the immediate cause of such disability is a very slowly reversible or irreversible tissue damage within the C.N.S. induced by the actions of the transient toxic substances mentioned above.

The respiratory difficulty induced by subjecting an animal to OHP is enhanced by successive exposures; this suggests that pulmonary damage is increased in such procedures. Herderer and Andre (1940) report that the lungs become more



susceptible to the damaging action of OHP with repeated exposures. But this enhancement of the respiratory response may also be interpreted as due to effects on either central or peripheral nervous mechanisms concerned with respiratory regulation. Be that as it may the respiratory difficulty seen during the stay in OHP and during the acute phase following decompression does not constitute a significant feature of the chronic phase of the post-decompressional reactions.

#### SUMMARY AND CONCLUSIONS

1. Young adult unanesthetized rats were subjected to O<sub>2</sub> (U.S.P.) at 65 pounds' pressure in short, single or successive exposures, for the purpose of studying recovery reactions. The chamber temperature was maintained at 24°C and the CO<sub>2</sub> absorbed. Decompression was carried out in stages to prevent bubble formation.

2. The reactions observed during the decompressional and post-decompressional periods were divided into *a*, acute, and *b*, chronic. The acute reactions occurred during decompression and immediately following decompression; they included various manifestations of C.N.S. involvement such as stages of stupor, coma, catatonia, hyperexcitability, hypotonia, and hypertonia, as well as tonic convulsive reactions, motor dysfunction and dyspnea. Although some animals possessed remarkable capacity for recovery from the acute effects, such recovery was rarely, if ever instantaneous or immediate, even after single exposures. Acute reactions persisted for various periods of from a few minutes to as long as several days after the animal's return to normal pressure, depending on the individual peculiarity and the severity of the exposure.

3. After apparent complete recovery from the acute reactions following a single exposure, subsymptomatic alterations persisted in the tissues for some time; that this is so was shown by the finding that successive exposures to OHP appropriately spaced so as to permit symptomatic recovery following each exposure, induced an additive effect which augmented the severity and prolonged the duration of the subsequent acute reactions.

4. The chronic effects were characterized by spastic motor paralyses which in the less severe cases involved only the fore legs but which in more pronounced cases affected almost the entire body musculature. These chronic effects were regularly induced by successive exposures (usually 3 per day); for the most part they developed gradually but occasionally they appeared after a single exposure. The spacing of the successive exposures was found to be an important determinant in the induction of the chronic effects.

5. Some degree of recovery from the chronic effects, or a compensatory readjustment, was observed in some animals over a period of weeks but only in minor cases did it appear to be complete. The chronic effects were predominantly permanent.

6. Animals showed a wide individual variation in their susceptibility to the induction of the chronic effects. Permanent motor paralyses were induced in some animals which had failed to show any evident acute reactions.

7. The occurrence of convulsive attacks or lesser disturbances during either the exposure to, or the decompression from, the OHP, was not essential to the induction of the permanent motor disabilities by successive exposures.

8. The susceptibility of the animals to the toxic action of OHP was progressively increased with successive exposures but this increase did not continue indefinitely; after having attained a plateau the susceptibility remained relatively constant unless the program of exposures was altered.

9. The reactions occurring in an animal during its exposure to, and decompression from, OHP, as well as the more transient of the acute post-decompressional reactions, would appear to be best explained as due to the toxic influence of an accumulation of endogenous  $\text{CO}_2$  in the tissues, increased tissue acidity and a direct action of OHP on tissue enzymes; the more persistent of the acute post-decompressional effects may be attributed to more slowly reversible alterations in the tissues, conceivably a slowly reversible tissue injury induced by these same transient toxic agents.

10. For an adequate explanation of the chronic post-decompressional effects, manifest by permanent motor disability, there is little choice other than that of an irreversible tissue damage and degenerative changes within the C.N.S., induced by the toxic agents referred to. The nature of the permanent disability points to the upper motor neurone as a part prominently affected, although the involvement of other portions of the C.N.S. is not ruled out.

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# THE CHLORIDE EQUILIBRIUM IN MUSCLE<sup>1</sup>

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The Boyle and Conway (1) theory of the state of electrolytes in muscle demands that a small quantity of chloride be present in the fiber water. This is contrary to the earlier evidence reviewed by Fenn (2) which suggested that all chloride is extracellular because of the similarity between the histological intercellular space and the "chloride space", that common water volume which all the chloride of muscle must occupy if it is to have a uniform concentration equal to that in plasma water. The Conway (3) group found evidence of "fiber" chloride in the fact that the chloride space of frog sartorii exceeded the inulin space determined after immersion of the muscle in inulin-Barkan solution. On analyzing isolated washed frog muscle fibers, Dean (4) found very little chloride, but Heilbrunn and Hamilton (5), in a hot water extract of isolated fibers, found probably more chloride than is demanded by the Boyle and Conway theory.

In excised muscles immersed in artificial media containing very high concentrations of potassium, Boyle and Conway (1) found the content of fiber chloride estimated by means of inulin space to vary according to their theory. Increasing quantities of potassium in the external medium are supposed to carry chloride and other diffusible anions into the fibers as the potassium salt through membrane pores as a Donnan equilibrium (see Wilde, 6). Since at equilibrium the KCl salt will have attained a given concentration in a certain volume of fiber water, an increase in the latter volume, as imposed by hypotonicity of the external medium, must carry an additional KCl content inside the fibers. A mild change of either type is predicted to produce changes hardly detectable with present analytical methods, but application of both simultaneously should cause a detectable change.

This has been tested in live nephrectomized rats by injecting potassium in doses sufficiently mild or physiological that the rats could at least walk about. Fiber chloride was measured as the difference between chloride and inulin space multiplied by plasma chloride concentration. Special modifications of recent analytical methods for inulin and chloride provided analysis of aliquots from a common zinc filtrate prepared from a single sample of muscle. Accuracy was further improved by application of a colorimetric method for inulin which gives a negligible control "blank" for muscle.

**PROCEDURE AND METHODS.** Two albino rats of the same litter and sex were nephrectomized under ether by retroperitoneal approach. Rat A received intraperitoneally 66 cc. per kilo of a solution containing 3.75 grams per cent inulin or equivalent sucrose, 150 mM Na, 125 mM Cl, and 25 mM bicarbonate;

<sup>1</sup> Part of this work was summarized in *Science* **98**: 202, 1943.

in rat B, 50 mM K replaced equivalent Na in the above solution and only this rat had access to water during the 22 hours' equilibration.

After anesthesia with Na amytal, 100 mgm. per kilo injected under the shoulder skin, the rat was bled from the cut throat over a funnel with the aid of massage of limbs and abdomen. The plasma samples and hematocrit ratio were obtained by centrifugation of the blood thus collected in a 15 cc. graduated tube containing anticoagulant ammonium oxalate, 2 mgm. per cc. of blood.

Inulin and chloride in minced muscle were measured in a Somogyi zinc filtrate after addition of  $\text{NaNO}_3$  such that the  $\text{NO}_3/\text{Cl}$  ratio was about 50. While the nitrate presumably rendered any "adsorbed" chloride "free" by anionic exchange it also peptized a muscle substance which gave an interfering green color in the Seliwanoff color reaction used for the colorimetric determination of inulin as fructose. This difficulty was obviated by adding the nitrate *crystals* after removing the very small volume of filtrate needed for the determination of inulin. The great bulk of filtrate used for chloride was concentrated by evaporation before adding the acidified silver nitrate for the Van Slyke wet ash procedure. The end point of the Volhard titration was very distinct and the yield exceeded that from wet ashing by 38 per cent of itself, a yield similar to that from the hot water extract of Heilbrunn and Hamilton (5), who first called attention to this defect in the digest procedure for muscle. Repeated determinations were much more uniform in yield than were those done by digestion.

The detailed procedure followed in the analysis for inulin, chloride, water, potassium, and fat is as follows:

Strip only white sheet muscles from both thighs of the rat into slender pieces. Remove all visible connective tissue, fat, and blood. Store the strips in a glass covered bottle during this processing. Finally mince them to a pulp with scissors and mix thoroughly on a glass plate.

Determine inulin and Cl of muscle from 4 grams of mince placed in a 100 cc. porcelain mortar. Grind the muscle thoroughly in 4 grams of Cl-free sand. Since aliquots of filtrate for determination of inulin and Cl are required, subsequent additions of fluid reagents must be quantitative and in sum equal to 100 cc. Add 4 cc. of a mixture of 0.5 N  $\text{H}_2\text{SO}_4$  and 0.5 M  $\text{ZnSO}_4$  and grind to a paste. Mix in 4 cc. of aqueous NaOH of such strength that 1 cc. of zinc sulphate solution containing phenolphthalein is permanent pink on addition of 1.2 cc. of NaOH. Add with stirring 92 cc. of redistilled, Cl-free water. Let stand covered one hour with occasional stirring. Transfer to a glass-stoppered Erlenmeyer flask washing in all the brei with supernatant fluid. Let the brei settle until several cubic centimeters of clear supernatant may be decanted for the inulin sample. Filter the latter repeatedly until clear through Sargent no. 501 filter paper. Take 2 cc. of filtrate for the Seliwanoff color reaction for inulin or sucrose as fructose as developed by Steinitz (7). Return any excess filtrate to the flask for subsequent Cl analysis.

Determine inulin and Cl of plasma in aliquots of a single zinc filtrate. Pipette 1 cc. of plasma into a 50 cc. volumetric flask along with about 30 cc. of Cl-free water. Add separately with shaking 1 cc. each of the zinc and hydroxide solutions. Add water to the mark, shake, let stand, and then remove and filter slightly more than 2 cc. of the clear fluid for the inulin analysis. Dilute 2 cc. of this with 4 cc. of water and carry 2 cc. of the diluted material through the inulin color reaction simultaneously along with the muscle sample and a suitable inulin standard. Measure the light absorption of these consecutively in the same tube in a Klett-Summerson photoelectric colorimeter using filter no. 50. (The performance of stock filters varies one to the other. Choose a filter which gives maximum absorption.)

The concentration of inulin in the three samples is about 0.04 mgm. per cc., within 10 per cent of each other, of that absolute concentration and range of concentration found by Steinitz to obey Beer's law.

Muscle blanks from 4 rats average 4.6 colorimeter scale units, which is 5.7 per cent of the average experimental inulin reading, 81.0. The range of distribution of the blank values is 3.3 per cent of 81.0. Subtract the blank value from each muscle inulin reading and correct also for the plasma blank, 0.56 scale unit.

In calculating the inulin space of muscle consider that the inulin in the colorimeter tube has for muscle been diluted by the water of the muscle sample as well as by the 100 cc. of water and reagents added and that the inulin of the 1 cc. of plasma has been diluted to 150 cc. considering the 2 stages of dilution. Inulin space in liters per kilo of fresh muscle is equal to inulin content of muscle divided by inulin content per liter of plasma water. With muscle and plasma inulin samples so closely matched, inulin contents become directly proportional to colorimeter readings and we write inulin space of muscle equal to

$$\frac{(1000/\text{gram muscle sample})(100 + \text{gram sample water})(\text{Klett reading})}{(1000/\text{gram water in 1 cc. plasma})(150)(\text{Klett reading})}$$

To complete the analysis for muscle Cl, place 340 mgm. of Cl-free  $\text{NaNO}_3$  crystals in the Erlenmeyer flask, shake, and let stand overnight. Filter and concentrate by evaporation the filtrate measured 25 cc. at a time into a 50 cc. Pyrex centrifuge tube with short conical bottom. Heat the tube in a water bath and further accelerate evaporation by circulating over the fluid air dried by bubbling through concentrated sulphuric acid. After about 80 cc. of filtrate has been concentrated to about 3 cc. carry out Van Slyke's analysis (8) for Cl by digesting the filtrate 20 minutes with nitric acid-silver nitrate. The heat of digestion coagulates the AgCl precipitate and so improves the end point of the Volhard titration. Further sharpness results from chilling the tubes and centrifuging down the precipitate just before the end point.

If the yield of chloride from this  $\text{NaNO}_3$ -zinc filtrate is used as a standard of comparison, the yield from the Van Slyke digest is 38 per cent lower, that from the plain zinc filtrate is 15 per cent lower, and that resulting from multiple extraction of a zinc precipitate with  $\text{NaNO}_3$  solution is 6 per cent higher. Tests run with blanks and knowns verify the following: that the redistilled water and all reagents are Cl-free; that in the evaporation or concentration process there is no loss due to heat or contamination from the circulated dry air; that the presence of  $\text{NaNO}_3$  does not affect the value of knowns. The muscle chloride values derived are probably more than 6 per cent below the true values.

Continue the analysis for plasma Cl as for muscle by adding 170 mgm.  $\text{NaNO}_3$  crystals to the volumetric flask. The final filtrate measures about 35 cc. and is concentrated in one stage in the 50 cc. tube. Plasma Cl by this method exceeds that from direct digestion rather uniformly by 4.4 per cent.

Chloride space in liters per kilo of fresh muscle is equal to m Eq. of Cl per kilo of muscle divided by m Eq. of Cl per kilo of plasma water. The Donnan ratio is ignored in this calculation. With the concentration of protein and associated but ionized sodium different in the three extracellular phases of muscle, vascular, lymphatic, and intercellular, it seems probable that the chloride concentration because of the Donnan effect is different in each. With concentrations and volumes in two of these phases indeterminable, it seems futile as is often done to represent chloride contents summed for all three as identical to that of an equal volume of protein-free fluid equilibrated against plasma. Since plasma volume is taken to be the largest of the three phases (Fenn, 2), it seems sufficient to represent the concentration in all three by that in plasma water.

Although the careful stripping and examination of the muscle bundles eliminates blood contained in any visible blood vessels, the chloride present in any erythrocytes otherwise remaining in the tissue is ignored. Many such difficulties are presumably obviated in part by the use of the control rats A.

Determine muscle water and potassium from one gram of mince in a covered platinum crucible: water content as weight loss overnight at 105°C. in an electric oven; potassium from the residue wet with dilute sulphuric acid, dried, and ashed 8 hours in an electric muffle furnace heated slowly up to and maintained at 575°C. as tested each time with Seger pyrometer cones. Analyze for K by a modified Shohl and Bennett chloroplatinate method (9, 10).

Determine plasma water and potassium from 1 cc. of plasma in a platinum crucible treated as above for muscle.

The neutral fat of the muscle mince from four B rats, measured according to Buell (11), averages 1.9, with a range of 0.5, per cent of the dry weight of muscle. The average weight of a kilo of fresh mince, when dried, is 0.25 kgm. All measurements in terms of dry weight are referred to this figure. The small error, 0.5 per cent, due to variation in the fat content of the dry mince is ignored.

TABLE 1

*Water phases of sheet thigh muscles in nephrectomized rats (liters per quarter kilo of dry muscle) 24 hours after injection of solution A (no potassium) into litter mate A and of solution B (high potassium) into mate B*

Extracellular values  $E_i$  and intracellular values  $I$  determined from the partition of inulin;  $E_{Cl}$  from chloride.  $E_i$  for "Rat" calculated from the quantity of injected inulin and expressed as liters per kilo of rat. " $\Delta$  Wt." of "Rat" equals weight loss of the rat minus dried feces voided, as per cent of initial body weight after nephrectomy.

PAIR NO.	LITTER MATE A					LITTER MATE B				
	Muscle		Rat	Muscle		Muscle		Rat	Muscle	
	$E_{Cl}$	$E_i$	$E_i$	$I$	$\Delta$ wt.	$E_{Cl}$	$E_i$	$E_i$	$I$	$\Delta$ wt.
1	0.228	0.169	0.289	0.592	-3.7	0.212	0.155	0.281	0.655	-1.5
2	0.228	0.166	0.253	0.635		0.241	0.149	0.216	0.707	
3	0.162	0.133	0.396	0.630	-2.7	0.219	0.143	0.310	0.703	-0.8
4	0.178	0.122		0.612	-1.0	0.171	0.101	0.284	0.691	-2.8
5	0.125	0.114		0.628		0.178	0.133	0.290	0.690	
6	0.203	0.140	0.322	0.622	-6.0	0.198	0.120	0.430	0.720	+7.0
7	0.153	0.109	0.305	0.629	-2.6	0.194	0.129		0.684	+2.4
8	0.153	0.115	0.395	0.615	-4.5	0.183	0.137	0.356	0.642	-2.6
9	0.163	0.150		0.600	-6.4	0.150	0.100		0.710	-0.2
Mean	0.177	0.135	0.327	0.618	-3.84	0.194	0.130	0.310	0.689	+0.21
$\sigma$		0.023	0.051	0.018	1.80		0.016	0.060	0.027	3.21

" $E_i$ " for pair no. 2 determined from sucrose; rat 8 B given no water to drink.

**RESULTS AND DISCUSSION.** *The inulin space.* The interpretation of the data presented depends upon the validity of the inulin space as a measure of the extracellular space  $E$  of rat muscle. Measurements of the inulin remaining in the peritoneal water found in some rats demonstrate that the passage of the inulin to the plasma had reached equilibration. Preliminary comparison between  $E$  derived from inulin and from the much smaller molecule sucrose is available. The  $E_i$  of muscle measured with sucrose in rat pair 2 of table 1 is almost identical to that of their litter mate pair number 1 measured with inulin.  $E$  values for an entire rat, as measured from the quantity of inulin injected, are also given in table 1. In rats A the average  $E$ , 0.327 liter per kilo minus 0.066 liter, the

quantity of isotonic sodium salt injected, leaves 0.26 liter, the initial value. This approximates values by other methods for laboratory animals as listed in the literature. Inulin was used in all the other rats. It develops more color per molecule than sucrose in the Seliwanoff reaction and can therefore be used with less osmotic distortion of the E space.

*Chloride compared to inulin space.* E calculated from chloride exceeds E from inulin in either rats A or B (table 1). In the t test (Treloar, 12) for the significance of the difference between paired values the probability P is less than 0.0006 in either rat group that such a difference could arise in random samples from a common pool. The differences ( $E_{Cl} - E_i$ ) when averaged separately for rats A and for rats B also show means significantly different (P, 0.018). The E spaces for inulin and for chloride are not only different in an absolute sense but their difference changes functionally in passing from group A to B.

This is attributed to the entrance of more chloride into the muscle fibers or non-inulin space of rats B than of rats A. The chloride content of muscle fibers,  $Cl_1$ , per quarter kilo of dried muscle, rises from 4.54 to 6.98 m Eq. (table 2). This increase of 2.44 m Eq. is significant by t test (P, 0.017) and represents a rise of 53.7 per cent over the A value for fiber chloride.

*Fiber chloride in relation to fiber water volume and plasma potassium.* According to the Boyle and Conway theory, the chloride content of muscle fibers is referred to

$$Cl_1 = Cl_i I = (Cl/d) K I. \quad (1)$$

$Cl_1$  refers to the concentration of chloride in fiber water of volume I and the substitution in the last member comes from equation (1) of Wilde (6):  $Cl_i = (Cl/d) K$ , in which  $d$ , the sum of all diffusible anions, and  $Cl$  and  $K$  are all concentrations in extracellular water.

The water content of the B rat fibers exceeded that of the A fibers (table 1) by 0.689 - 0.618, or 0.071 liter per quarter kilo of dry muscle. The probability that the difference is insignificant is less than 0.0001 and the change represents an increase of 0.071/0.618, or 11.5 per cent of the A fiber water. Part of the increment arose from the drinking water allowed only the B group. A comparison of the percentage change in body weight in groups A and B ( $\Delta Wt.$ , table 1) suggests this. The probability is 0.019 that the difference in body weight change is insignificant. Rat B 8 received no drinking water and showed only half the average fiber water increment; rat B 1 may not have drunk. If Boyle and Conway theory applies (1, p. 17), the remainder of the water taken up by the B fibers came in part from water injected as the isosmotic potassium salt ( $\frac{1}{2}$  of the B solution). Since this salt is assumed to enter freely into the fibers it has no control over water. Water injected with potassium is supposed to act just as water alone and to enter the I and E phases in proportion to their original water content. In consideration that the volumes of solution injected into the two groups were equal, the difference in behavior of water anticipated would be that expected if the B rats lost sodium from E in equivalents equal to the potassium injected. In this connection the fibers of rat B 8, which drank no water, took up water equal to half the average increment found in rats which did drink.

The predicted chloride increment due solely to water uptake by the B fibers is seen to be  $(\overline{Cl}_1)_B \Delta I$ ,  $10.12 \times 0.071$ , or 0.718 m Eq. per quarter kilo of dry muscle. That amounts to  $0.718/2.44$ , or 29.5 per cent of the observed increase in fiber chloride. Without this increment relegated to water uptake the remaining 70.5 per cent of the change in content of chloride, that related to the elevated plasma potassium, would have been barely significant ( $P$ , 0.079). Unfortunately the water contents of the fibers within the groups A and B are not scattered over a wide enough range for calculation of a reliable correlation coefficient between  $I$  and  $Cl_1$ .

The plasma potassium values are sufficiently scattered to warrant application of a gross correlation. The correlation  $r$  between  $K$  and  $Cl_1$  (table 2) in rats A

TABLE 2

*Electrolytes in nephrectomized rats 24 hours after injection of solution A (no potassium) into litter mate A and of solution B (high potassium) into mate B*

$Cl$  and  $Cl_1$ , m Eq. of chloride per kilo of water in plasma or muscle fibers respectively;  $K$ , m Eq. of potassium per kilo of plasma water.  $Cl_1$  (in boldface type), m Eq. of chloride in the muscle fibers associated with a quarter kilo of dry muscle.

PAIR NO.	LITTER MATE A				LITTER MATE B			
	$Cl$	$K$	$Cl_1$	$Cl$	$Cl$	$K$	$Cl_1$	$Cl$
1	10.7	7.0	6.4	107.7	10.3	10.7	6.7	118.0
2	9.0	8.5	5.7	92.0	12.9	14.7	9.1	98.6
3	4.9	6.9	3.1	107.0	12.1	12.4	8.5	111.3
4	9.0	10.1	5.5	98.0	11.2	15.9	7.7	111.0
5	1.9	7.4	1.2	109.3	7.0	11.8	4.8	107.2
6	13.2	14.9	8.2	98.4	11.6	14.2	8.4	108.4
7	8.0	8.1	5.0	112.4	10.0		6.9	106.7
8	7.0	8.3	4.3	112.3	8.2	10.8	5.3	115.3
9	2.5	8.6	1.5	113.2	7.7	8.8	5.4	108.3
Mean	7.35	8.87	4.54	105.6	10.12	12.41	6.98	109.4
$\sigma$	3.51	2.32	2.16		2.07	2.22	1.45	

is 0.617. The probability  $P$  that this figure arises from uncorrelated material, as tested by the application of the "analysis of variance" adapted to a table of  $t$  (12, p. 171), is 0.076. In the B rats  $r$  is 0.777 with a  $P$  value of 0.024. For the combined rats  $r$  is 0.647 and  $P$  is 0.005. While such a correlation affirms the validity of the Boyle and Conway concept, it does not deny the possibility of the adsorption of chloride as the potassium salt.

Unfortunately the overall relation  $Cl_1 = (Cl/d) K I$  is hyperbolic rather than linear and so cannot be fitted to the technic of "partial correlation" to test the relative importance of changes in  $I$  and in  $K$ .

*Concentration of fiber chloride in relation to plasma potassium.* Stronger confirmation of the Boyle and Conway concept comes from correlating the concentration of plasma potassium  $K$  with  $Cl_1$ , the concentration of chloride in fiber water (table 2). If chloride in muscle is adsorbed at immobile valence points as



the salt of potassium, in consideration of the small quantity of chloride at present relegated to the fibers, it seems unlikely that it would have any domineering osmotic control over water. In an adsorption complex  $K$  should have no relation to chloride in fiber water, to its concentration  $Cl_1$  in fiber water. The Boyle and Conway theory demands this relation. Among rats A,  $r$  is 0.602 with  $P$  equal to 0.041; in group B,  $r$  is 0.685 and  $P$  is 0.061; for the combined rats,  $r$  is 0.710 and  $P$  is 0.0014.

From the combined matched values from rats A and B the regression line,  $Cl_1 = 0.28 + 0.795 K$ , has been fitted. This is analogous to equation (1) of Wilde mentioned above:  $Cl_1 = (Cl/d) K$ . According to Boyle and Conway (p. 17) as interpreted by Wilde (6, p. 110), when  $K$  is elevated by substitution of potassium for sodium or  $N$ , in E, as was done in the B rats, E shrinks for reasons mentioned above. Thus while E loses some of all anion types along with potassium to the fibers, it also loses water to the fibers in such a way as to keep  $Cl$ ,  $d$ ,  $K_1$ , and  $N + K$  constant. Therefore  $Cl_1 = (Cl/d) K$  in this case is the equation of a line and one may set  $Cl/d = 0.795 = 107.5/d$ . Thus from  $Cl = 107.5$ , the average value for plasma chloride from all rats,  $d = 135.2$ , and  $d - Cl = 27.7$ , a very likely value for diffusible anions other than chloride in plasma, particularly bicarbonate.

*Fiber chloride in relation to plasma chloride.* From Boyle and Conway according to equation (9) or (10) of Wilde (6), a linear relation is to be expected between  $Cl_1$  and  $Cl$ :  $Cl_1 = D - (E/I) Cl$ . This would apply only within groups A or B within which no water shifts were known to occur. Unfortunately a measure of correlation is vitiated by the wide variation of plasma chloride among the individual rats. In rats A,  $r$  is  $-0.548$  and  $P$  is 0.13; in rats B,  $r$  is  $-0.124$  and  $P$  is 0.56; in the combined groups,  $r$  is  $-0.308$  and  $P$  is 0.21.

By various theories of adsorption or ionic exchange of chloride between the fibers and interstitial water or plasma, it would be expected that the content of chloride per muscle fiber would be related to the plasma chloride concentration  $Cl$ . This is on the basis that chloride be adsorbed as such, alone, not along with certain cations. It can be assumed that a given group of fibers will have definite association with a given quantity of dry residue in muscle, regardless of water exchanges, that chloride per unit fiber is accurately represented by chloride per quarter kilo of dry muscle,  $Cl_1$ . The correlation,  $Cl_1$  to  $Cl$  (table 2), has been tested, again with the disadvantage of wide individual variation in  $Cl$ . In rats A,  $r$  is  $-0.49$  and  $P$  is 0.21; in group B,  $r$  is  $-0.44$  and  $P$  is 0.20; in the combined rats,  $r$  is  $-0.226$  and  $P$  is 0.36. The correlation is negative, rather than positive as demanded for adsorptions, and insignificant.

*Fiber potassium.* In a simplified view of the Boyle and Conway concept (6) the potassium of muscle fibers is considered divided into two phases: 1, " $K_1$  is fixed, except for exchange with other cations, to non-diffusible organic acid radicals Y and 2, ' $K_1$  is an inorganic salt of chloride, bicarbonate, etc., ' $K_1 d_1$ , freely diffusible as the salt and mixing with  $K$  in the plasma so that at equilibrium ' $K_1 = K$ . With the elevated  $K$  of rats B either the salt ' $K_1 d_1$  or the adsorbed " $K_1$  or both may increase in content in the fibers. The content in m Eq. of

the particular type of potassium in a given group of fibers is represented as potassium per unit dry weight of muscle and is symbolized in bold face type.

$\mathbf{K}_1$  is significantly larger in the B rats (table 3;  $P, 0.0002$ ). An average of 10.5 m Eq.  $K$  entered the fibers associated with a quarter kilo of dry muscle residue.  $\mathbf{K}_1 - \mathbf{K}_1 = \mathbf{K}_1$  is also significantly larger in the B rats ( $P < 0.0002$ ). Besides the potassium entering the fibers as  $K d$ , according to Boyle and Conway, some additional potassium, 8.8 m Eq. per quarter kilo dry muscle, has become associated with organic Y radicals possibly in exchange for other cations (Steinbach, 13; Heppel, 14) such as sodium.

In spite of these increments the concentration of potassium in fiber water  $K_1$  (table 3) fell 7.9 mM ( $P, 0.106$ ). As mentioned above according to the Boyle and Conway theory, had the B rats drunk no water, potassium and water would have entered the fibers in such proportion as to keep  $K_1$  unchanged. To follow this rule,  $(\mathbf{K}_1/I)_A = (\mathbf{K}_1/I)_B$ ,  $134.4/0.618 = 144.9/I_B$ ,  $I_B$  would equal 0.666 which approximates the value 0.642 for rat B 8 which drank no water (table 1). The apparent 7.9 mM dilution of  $K_1$  in B fibers may have originated in the water drunk.

Part of the potassium in muscle or in plasma may be inactive or non-ionized. The ratio  $K Cl/(K_1 Cl_1)$  listed in table 3 averages 0.55 for A rats instead of 1.0 as required by the Donnan membrane equilibrium. If for the A rats one writes  $(K Cl/Cl_1)/K_1 = (K Cl/Cl_1)/217.6 = 0.55$ , it is evident that for the ratio to equal 1.0,  $K_1$  must be 119.7 instead of 217.6 or 55 per cent active. If the activity of the plasma potassium, as is certainly true (15), is below 1.0, the activity of  $K_1$  is even less than 0.55. Whether Y K is sufficiently mobile and free in muscle water to affect the activity of potassium at the fiber surface is another question.

Were  $K_1$  completely active as the salt K Y and exerting the average osmotic value 300 mM known for mammalian tissues, the valence of Y would approximate 2.5.

From tables 3 and 1, respectively, we write the ratio of the average changes  $\Delta\mathbf{K}_1/\Delta I = 10.5/0.071 = 148$  mM. It appears that potassium entered the fibers as an isotonic solution as observed by Fenn (16). This is predicted from the Boyle and Conway theory even though the potassium enters as the dilute salt  $K d$  distributed throughout the fiber water. With potassium substituted equivalently for sodium in the external medium of the B rats, water and potassium, as explained above, would enter the fibers so as to keep  $K_1$ , and therefore  $\Delta\mathbf{K}_1/\Delta I$ , constant and equal to  $N + K$ . Since the B rats drank water it is surprising that the calculated value 148 mM approximates the classical value 150 as nearly as it does.

*Potassium compared to sodium in support of E space.* If extracellular potassium in contrast to sodium has no osmotic control over water, the value of E should be smaller in the B than in the A rats. Unfortunately the absolute reduction expected because of this was counteracted by the water drunk by the B rats. Also the large volume of fluid required as a vehicle for the solution of inulin, when injected, caused such an expansion of E in both groups as to diminish the

calculated *percentage* change. Suitable calculations resolving these difficulties predict the theoretical change for an entire rat, A to B, to be  $-0.006$  liter per kilo. The average difference calculated from the injected inulin (table 1) is  $-0.017$  ( $P > 0.56$ ) or  $-5.2$  per cent of  $E_A$ . The average difference in E per quarter kilo of dry muscle is  $-0.005$  liter ( $P > 0.56$ ) or  $-3.7$  per cent of  $E_A$  (table 1). For a kilo of fresh or wet muscle  $\Delta E$  is  $-0.017$  liter ( $P, 0.05$ ) or  $-12.5$  per cent of  $E_A$  (data not tabulated). In the last calculation the larger proportion of water in the B fibers tended to "dilute" E as calculated for fresh muscle. The moderately good agreement between the percentage change of

TABLE 3

*The potassium of sheet thigh muscles of nephrectomized rats 24 hours after injection of solution A (no potassium) into litter mate A and of solution B (high potassium) into mate B*

$K_1$ , m Eq. potassium per kilo of muscle fiber water.  $K_1$ , fiber potassium in m Eq. per quarter kilo of dry muscle. " $K_1$ ", that  $K_1$  associated only with Y of Boyle and Conway (see text).  $KCl/K_1Cl_1$ , the Donnan ratio further described in the text.

PAIR NO.	LITTER MATE A				LITTER MATE B			
	$K_1$	$KCl/K_1Cl_1$	" $K_1$ "	$K_1$	$K_1$	$KCl/K_1Cl_1$	" $K_1$ "	$K_1$
1					204	0.55	140	151
2	216	0.40	132	137	211	0.54	140	146
3	229	0.65	137	142	218	0.73	139	150
4	220	0.50	128	136	205		133	141
5	219		129	133	202	0.66	136	146
6	218	0.58	125	135	222			
7	217	0.52	124	136	221	0.69	135	142
8	209	0.64	123	128	195	0.63	132	138
9	213		123	128				
Mean	217.6	0.55	127.6	134.4	209.7	0.63	136.4	144.9
$\sigma$	6.33		5.27	3.53	10.3		5.21	2.66

E in dry muscle and in the entire rat suggests that the inulin had become well dispersed or equilibrated in the extracellular space.

Along with the expanded E of the A rats should appear also an enlarged blood volume. The average difference in the volume of blood "milked" from the cut throat of A and B rats amounted to 2.67 minus 2.38 or  $-0.29$  per cent of the initial body weight ( $P, 0.28$ ). The averaged hematocrits in A and B rats were 47.3 and 47.4 per cent corpuscles respectively.

I am indebted to Dr. W. O. Fenn, whose discussion in Boston of Dr. Heilbrunn's paper (5) led to my use of nitrate in the chloride analysis; to Dr. J. R. Doty who made a color absorption curve with the Coleman spectrophotometer for the Seliwanoff reaction for inulin and who made helpful suggestions bearing on the successful preparation of a muscle filtrate of inulin; and to Sarah Jane Braniff, who did the fat analyses.

## SUMMARY

The chloride of muscle fibers in live rats is estimated by comparison of the distribution of inulin and chloride, which are analyzed accurately in aliquots of the *same* zinc filtrate of muscle. In the filtrate of controls the blank for inulin, if measured by the Seliwanoff color reaction, is negligible. The distribution of inulin in muscle is identical to that of sucrose, a much smaller molecule. The yield of chloride is improved by filtering in the presence of excess nitrate anions and the end-point of the Volhard titration in the zinc filtrate is unusually distinct.

Physiological conditions predicted by Boyle and Conway (1) to increase fiber chloride, tried to date only on excised muscle, are now tested in live rats: an 11.5 per cent increase in fiber water and an elevation of plasma potassium from 8.9 to 12.4 mM. The chloride content of the fibers rises significantly from 4.54 to 6.98 m Eq. per quarter kilo for dry muscle. Thus the distribution of chloride is not a reliable measure of functional *change* in the volume of extracellular fluid much less of its absolute value. This chloride content has an insignificant but negative correlation,  $-0.226$ , to plasma chloride concentration. This favors a Boyle and Conway but disallows an adsorption theory involving the adsorption of chloride alone. Whether the chloride content is related to the water content of fibers is uncertain, but a significant correlation to the concentration of plasma potassium exists. There is likewise significant correlation between the *concentration* of fiber chloride and of plasma potassium. This again is disallowed by an adsorption but demanded by a Boyle and Conway theory. The variables fit with significance the linear equation derived by Wilde (6) from Boyle and Conway:  $Cl_1 = (Cl/d) K$ , in which the constant  $(Cl/d) = 0.795$  estimated from the regression line of the data gives a reasonable value to  $d = Cl + HCO_3 = 107.5 + 27.7$  in plasma.

The fibers gain potassium as it "appears" to enter them as an isotonic solution, 148 mM, in the water increment as demanded by Boyle and Conway. If a Donnan equilibrium in a fiber having a membrane with ion pores is to apply, the activity of fiber potassium must be as low as 0.55.

That extracellular potassium, because of ready penetration into muscle fibers as an inorganic salt, cannot according to Boyle and Conway support extracellular fluid osmotically as does sodium, was confirmed by measurement by means of inulin of the fiber water content and of the extracellular fluid volume of muscle and of entire rats. The changes in extracellular fluid though in the predicted direction were for technical reasons insignificant.

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# DENERVATION ATROPHY OF BONE AND MUSCLE. AN EXAMINATION OF THE EFFECT OF CHOLINE AND SOME FURTHER OBSERVATIONS ON THE METABOLISM OF PHOSPHORYLCHOLINE AND DEPOSITION OF P<sup>32</sup> IN BONE<sup>1</sup>

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The established relationships of choline to fat and protein metabolism suggested the extension of our studies on choline metabolism (1, 2) to an investigation of the effect of choline on the so-called atrophy of disuse of muscle and bone resulting from denervation. This examination was further prompted by preliminary data which were interpreted to mean that choline has some rôle in mammalian bone metabolism (3). Although choline has been known for some time to play a rôle in avian bone metabolism (4), no attempt appears to have been made to link this substance to the nutrition of mammalian bone.

While a number of drugs and accessory food factors have been submitted to test for their possible therapeutic usefulness in denervation atrophy of muscle (5, 6), the list is not extensive. Published studies on atrophy of bone following denervation in experimental animals are also limited (7-9). The work reported in the first section of this paper describes the effect of parenteral choline administration on the denervation atrophy of bone and muscle of the forelimbs of the rat, resulting from section of nerves of the brachial plexus.

The work presented in the latter part of this paper is an extension of our earlier studies on the metabolism of phosphorylcholine containing the radioactive phosphorus isotope (3, 10). A highly potent sample of radioactive phosphorus had become available at the time we were engaged in denervation atrophy experiments. As we were anxious to extend our earlier, preliminary observations on the metabolism of phosphorylcholine, the animals on the denervation atrophy experiment were utilized for this purpose. This arrangement also provided an excellent opportunity for examining the effect of denervation atrophy on the uptake of radioactive phosphorus by bone.

**METHOD.** A group of 41 male albino rats weighing between 140 and 200 grams each were divided into four groups of about equal number, each group containing a representation of the weight range (see table 1). The nerves of the brachial plexus of two groups were sectioned unilaterally at the axillary level under nembutal anesthesia. A portion of nerve fiber was removed to insure against any regeneration during the experimental period. The second two groups were incised, but the nerves not sectioned, to provide a group of sham-operated con-

<sup>1</sup> The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the University of Rochester.

trols. To avoid possible errors due to differences in the weight of opposite tissues an equal number of operations were made on right and left legs.

One group of sham-operated animals and one group of rats in which the brachial nerves had been sectioned received daily intraperitoneal injections of 10 mgm. of choline chloride in isotonic saline (approximately 60 mgm. per kgm.). After twenty-one days the injections were stopped. The anterior portion of the animals was x-rayed on the twenty-second day, and they were sacrificed on the twenty-fourth day. Throughout the experiment the rats were maintained on a stock Purina fox-chow diet, food and water being provided *ad libitum*.

Twenty-four hours prior to sacrificing, half of each of the four groups were given a trace dose (approximately 0.00065 mgm. per gram of rat) of radioactive phosphorylcholine.<sup>2</sup> The remaining rats received radioactive inorganic phosphate. All administrations of tracers were made by the intraperitoneal route.

The animals were sacrificed by decapitation, and blood samples stabilized with heparin were obtained at this time. The red cells were separated by

TABLE 1  
*Body weight data on the rats employed in the denervation experiments*

TREATMENT (NO. RATS)	AVER. WT. AT START (RANGE)	AVER. WT. AT DEATH (RANGE)	WT. GAIN DURING EXPER. PERIOD
	grams	grams	grams
Sham (10).....	168 (151-187)	219 (174-268)	55
Sham + choline (10).....	160 (139-182)	226 (194-260)	65
Sectioned (11).....	166 (143-185)	231 (211-290)	65
Sectioned + choline (10)...	171 (143-201)	237 (180-270)	66

centrifugation as soon as possible and aliquots of the supernatant plasma retained for later analysis. At this point attention was turned to the carcass.

As representative samples of bone and muscle, we chose to examine the humerus and a group of flexor muscles which permitted rapid and reproducible dissection. A flexor group was chosen which consisted of carpi ulnaris, carpi radialis, palmaris longus, pronator teres and digitorus profundus. After sacrificing the animals, these muscles were removed from the forelimbs of each rat and immediately weighed as a group. The muscle sample from each limb was then dried 24 hours at 105° and reweighed after cooling.

Both humeri from each animal were removed, freed of connective tissue and extracted for consecutive 24-hour periods with alcohol and ether to remove water and fat. Fat-free dry weights were obtained, and the bones were individually

<sup>2</sup> From 8.5 mgm. of radioactive phosphorus supplied as an aqueous solution of phosphoric acid we were able to obtain 42.3 mgm. (47 per cent of theory) of calcium phosphorylcholine chloride (11). This preparation, at the time of synthesis, contained approximately  $1.5 \times 10^6$  counts per minute per milligram of ester on our Bale dipping type scale-of-four Geiger-Müller counters (12). For this experiment, a part of this preparation was dissolved in physiological saline and diluted to a suitable strength of radioactivity prior to administration.

We are indebted to Dr. Robley Evans of the Massachusetts Institute of Technology for the sample of radioactive phosphoric acid employed in the synthesis.

treated with boiling 3 per cent potassium hydroxide in ethylene glycol to remove organic material (13). The ash, after washing with boiling water, was dried at 120°C. to constant weight.

The individual bone ash preparations were dissolved in dilute hydrochloric acid and made to a suitable volume. One aliquot of this stock solution was measured for radioactivity and others taken for the determination of inorganic phosphorus (14). The alkaline solution of ethylene glycol and washings containing the organic material of bone were combined, made to volume and retained for radioactivity measurements.

RESULTS AND DISCUSSION. For purposes of clarity, the results and discussion of the effect of choline on denervation atrophy will be considered separately from

TABLE 2  
*Summarized data on muscle and bone of denervated and control rats*  
(Average  $\pm$  S.D.\*)

TREATMENT		WATER IN MUSCLE	ASH OF FAT-FREE HUMERUS	MUSCLE WET WEIGHT, OPER. SIDE AS % CONTR. SIDE	HUMERUS FAT-FREE DRY WT. OPER. SIDE AS % CONTR. SIDE	HUMERUS ASH WEIGHT, OPER. SIDE AS % CONTR. SIDE
		%	%			
Sham	Control side	74.86 $\pm$ 0.47	60.9 $\pm$ 0.82	100.1 $\pm$ 7.6	100.6 $\pm$ 1.7	100.8 $\pm$ 1.9
	Incised side	74.82 $\pm$ 0.50	61.1 $\pm$ 1.00			
Sham + choline	Control side	74.78 $\pm$ 0.28	59.4 $\pm$ 1.23	98.8 $\pm$ 6.7	98.5 $\pm$ 2.4	99.6 $\pm$ 1.8
	Incised side	74.80 $\pm$ 0.67	60.1 $\pm$ 1.23			
Sec-tioned	Control side	74.66 $\pm$ 0.46	60.2 $\pm$ 0.92	55.9 $\pm$ 6.4	91.7 $\pm$ 2.5	90.7 $\pm$ 3.9
	Oper. side	76.25 $\pm$ 0.84	59.6 $\pm$ 1.06			
Sec-tioned + cho-line	Control side	74.69 $\pm$ 0.45	60.4 $\pm$ 1.25	59.1 $\pm$ 9.2	91.3 $\pm$ 2.8	90.0 $\pm$ 1.3
	Oper. side	76.18 $\pm$ 0.76	59.6 $\pm$ 1.31			

\* S.D. = Standard deviation of the mean.

the results on the metabolism of phosphorylcholine and deposition of radioactive phosphate in bone.

*Choline and denervation atrophy.* Summaries of tissue weight changes are presented in table 2. Results on muscle and humerus have been expressed as weight of tissue on the operated side as a percentage of the control or unoperated side. Percentages of water in muscle and ash in the fat-free humerus are also given. Data on the body weights are given in table 1.

So far as the effect of choline on denervation atrophy of muscle and bone is concerned, significant differences were not noted in either muscle or humerus between sham-operated control and sham-operated choline-treated rats or between denervated control and denervated choline-treated animals.

Examination of the data which express the weight of the tissue from the operated side as a percentage of the control side (columns 4, 5 and 6, table 2)



convinced us that such ratios do not adequately describe the kind of weight changes which actually occur. Thus the calculation:—

$$\frac{\text{weight of tissue from operated side}}{\text{weight of tissue from control side}} \times 100 = \text{percentage,}$$

does not determine in which term (numerator or denominator) a change occurs when the determined percentage in one set of circumstances differs from that in another. In the experiments we have described, it was probable that the weight of the control forelimb of the unilaterally sectioned animals increased at the same time atrophic changes were occurring in the denervated limb. Hypertrophic growth in the control limb also required evaluation. As a means of evaluating separately such concurrent changes we have examined our data in the following way.

Muscle weight and humerus weight determined at autopsy have been plotted against body weight at death for the sham-operated animals, utilizing the data obtained from both limbs. Assuming a direct proportionality between body and tissue weights for the animals of the weight range employed, we have fitted a straight line to these data by the method of least squares and have thus obtained the normal body weight-tissue weight relationship. It is unlikely that the weights of these tissues are directly proportional to body weights, though from the data this simplification would appear justified for present purposes. Tissue weights from the operated limbs of the sectioned animals were compared with these data by plotting tissue weight from the operated limb at time of sacrifice against the weight of the animal at the *time of operation*. A graph of these data for the flexor muscles is shown in figure 1.

It is interesting to note that muscle weights at autopsy from the operated side of the sectioned animals (expt., operated side—fig. 1) when plotted against animal weights at the time of operation do not deviate appreciably from the expected muscle weights of normal animals of the same weights. This indicates that there was no significant *net* change in weight in the atrophic muscle by the twenty-fourth day after section. Thus, during the 24-day period of the experiment, our control rats gained on the average approximately 62 grams each; during the same period their normally innervated flexor muscles gained an average of 0.18 gram each (fig. 1). This gain of 0.18 gram is about 45 per cent of the final average muscle weight in the controls (0.40 gram). Since the weight of the muscle in the denervated limb was about 57 per cent that of the control limb (table 2), the muscle of the denervated limb must have weighed about the same at sacrifice as it did at the time of operation.

If an actual net loss of protein occurred, it must have been accompanied by a compensatory gain of lipid, since the water content of this muscle did not change greatly. It is unlikely that a compensatory increase of lipid occurred, as Hines and Knowlton (15) in similar experiments reported a constant percentage composition for total nitrogen.

The muscle of the contralateral unoperated limb apparently does not gain more mass than may be attributed to normal growth under the experimental conditions

(no hypertrophy). This is indicated by the fact that the weight of the flexor muscles from the unoperated limb of the unilaterally sectioned rats (expt., unoper. side—fig. 1) when plotted against their terminal body weight is the same as that for sham operated controls.

Fischer (16) showed that the reported favorable effect of atropine on denervation atrophy (5) was in reality due to cachectic inanition produced by atropine administration. Similarly, figures for the percentage difference between de-

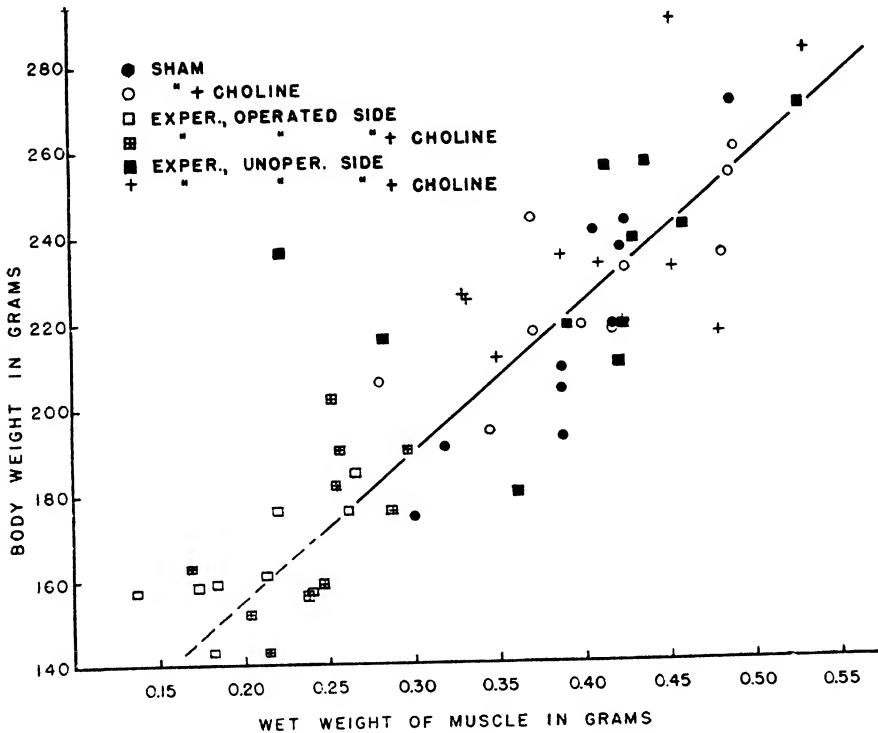


Fig. 1. Plot of terminal body weight against the weight of flexor muscles of the forearm. The equation for the line of best fit is  $y = 87.1 + 343.2x$ . Muscle weights from the operated side of the sectioned animals have been plotted against body weight at time of operation. There is approximately an equal distribution of □ (exper. oper. side) and ▣ (exper. oper. side + choline) above and below the trend line which has been extrapolated as a dotted line to include the lower points.

nervated and normal muscle reflect to a large extent normal growth changes rather than weight losses in atrophic muscle. That the latter observation was not made earlier is indeed surprising in view of the comment of Hines and Knowlton (15): "It seems that the rate of weight loss in the denervated muscle may bear some relationship to the growth rate of the species. The studies that have been made upon the rate of atrophy of corresponding muscles of the rat, rabbit, dog and human would indicate that for these species, at least, there appears to be a direct relationship between per cent loss of muscle weight in a given time and the growth rate."

A similar comparison for the humerus is presented in graphic form in figure 2.

When the weight of the humerus from the operated side of the sectioned animals (expt., oper. side—fig. 2) is plotted against animal weight at time of operation, not only has this tissue maintained its mass, but in contrast to muscle, considerable accretion has occurred during the period of the experiment.

The humerus of the unoperated limb apparently does not gain more mass than may be attributed to normal growth under these conditions (no hypertrophy). This is indicated by the fact that the weight of the humerus from the

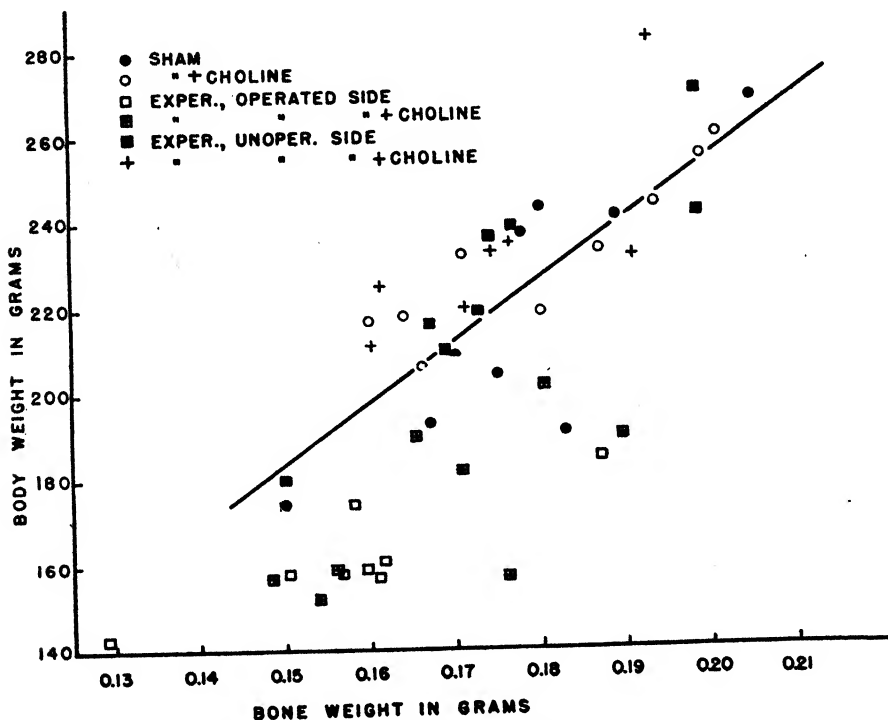


Fig. 2. Plot of body weight against the fat-free, dry weight of the humeri. The equation for the line of best fit, calculated from data for sham operated rats, is given by  $y = 1410x - 27.4$ . All of the values for the humeri ( $\square$  and  $\blacksquare$ ) fall below the trend line on the operated side.

innervated limbs of the unilaterally sectioned rats (expt., unoper. side—fig. 2) when plotted against their terminal body weight is the same as that for sham-operated controls.

Qualitative confirmation of these results was obtained from examination of the x-rays of these rats, made 21 days following operation. Striking differences in density were not apparent in the humeri of the brachial sectioned limbs as compared to the control forelimbs.

It may be noted from table 2 that there is a small increase in the percentage of water in the muscle of the operated side over the value for muscle from their unoperated side or over the sham controls. This finding is essentially in agree-

ment with that of Hines and Knowlton (15) for water changes in rat gastrocnemii 28 days after sciatic section. The percentage ash possibly decreases slightly in the sectioned limbs; however, the variation is too large to establish this conclusion. The range of ages of our animals undoubtedly contributed to the experimental scatter of ash percentages, as the percentage ash in bone increases considerably with age (17).

*Metabolism of phosphorylcholine and deposition of  $P^{32}$  in bone.* As we have shown above, no significant changes were discernible between choline-treated, sham-operated rats and their untreated controls or between choline-treated, denervated rats and their untreated controls. Accordingly, for purposes of the present discussion, grouping of our animals in regard to previous treatment with choline has been disregarded.

TABLE 3  
Summary of radioactivity data from denervation atrophy experiment  
(Average  $\pm$  S.D.†)

TREATMENT† (NO RATS)	P IN BONE ASH	SP. ACT. <sup>A</sup> ASH P	SP. ACT. OF BONE ASH OF OPER. SIDE AS PERCENTAGE OF CONTROL SIDE	PERCENTAGE OF ACTIVITY IN WHOLE BONE EXTRACTED BY GLYCOL	PERCENTAGE ASH IN FAT- FREE DRY BONE
	%		%	%	%
Sham + PO <sub>4</sub> <sup>o</sup> (8) . . . . .	16.5 $\pm$ 0.78	29.9 $\pm$ 4.18	98.1 $\pm$ 3.45	13.3 $\pm$ 2.37	60.2 $\pm$ 1.45
Sham + PCh* (8) . . . . .	16.5 $\pm$ 0.14	34.0 $\pm$ 3.37	99.9 $\pm$ 3.73	13.7 $\pm$ 1.51	60.5 $\pm$ 1.05
Sectioned + PO <sub>4</sub> (8) . . . . .	16.6 $\pm$ 0.14	28.4 $\pm$ 3.72	99.8 $\pm$ 3.32	15.4 $\pm$ 2.89	60.2 $\pm$ 1.07
Sectioned + PCh (8) . . . . .	16.2 $\pm$ 0.92	29.3 $\pm$ 2.64	95.1 $\pm$ 7.00	16.2 $\pm$ 2.64	59.7 $\pm$ 1.28

† S.D. = Standard deviation of the mean.

† Since we could not predict the effect of the previous choline treatment, half of the choline-treated rats were given inorganic phosphate and half were given phosphorylcholine. Similarly, half of the untreated animals received inorganic phosphate and the other half phosphorylcholine.

<sup>A</sup> Sp. Act. = Counts per minute per milligram of phosphorus.

<sup>o</sup> PO<sub>4</sub> = Inorganic phosphate.

\* PCh = Phosphorylcholine.

The method of preparation and analysis of our material has been presented previously. The results are summarized in table 3.

In general, large order differences between sham-operated animals given inorganic phosphate and those given phosphorylcholine are not apparent. The same is true for rats in which the brachial nerve had been sectioned. The specific activity of the phosphorus from the humerus is slightly higher in both sham and sectioned animals administered phosphorylcholine. While the difference is in the same direction as previously found (3), it is not as large and is probably insignificant. Such a small order difference can arise from experimental error in judging the activity equivalence of two different solutions containing radioactive tracers which are administered to different groups of experimental subjects.

Uncertainties of this nature can be largely obviated by expressing the activity

of the tissue in terms of the specific activity of the plasma phosphate, or of some phosphorus fraction of blood. Samples of blood plasma obtained from these animals were analyzed for phosphorus and radioactivity. While the activity of the plasma, in this instance, was too low to be entirely satisfactory, the average specific activity of the plasma of each of the four groups of rats was essentially identical (table 4). The plasma phosphorus values were also consistent between groups and therefore, it is unlikely that small differences in the values for the specific activity of the phosphorus of the bone ash are reflections of differences in the plasma.

The divergence of results between this and our earlier experiment on phosphorylcholine may be attributable to the dose of phosphorylcholine employed. Our preliminary data on bone were obtained on animals which received 0.17 mgm. of phosphorylcholine per gram of body weight. This dose, hardly to be considered a trace quantity, may have provoked detoxification mechanisms for its metabolism, or have so burdened the circulation with the handling of appreciable quantities of calcium and inorganic phosphate that this effect was mirrored in

TABLE 4

*Radioactivity data for plasma and humerus of control and experimental rats 24 hours after receiving phosphorylcholine or inorganic phosphate*

TREATMENT	PLASMA P	PLASMA P SP. ACT.	ASH P SP. ACT	PLASMA P SP. ACT. BONE ASH P SP. ACT.
	mgm. %			
Sham + PO <sub>4</sub> ....	13.2	471	29.9	15.7
Sham + PCh....	13.2	475	34.0	14.0
Sect. + PO <sub>4</sub> ....	13.1	470	28.4	16.5
Sect. + PCh....	13.1	497	29.3	17.0

the bone picture.<sup>3</sup> The results presented here, more adequately representing the results from trace-dose administration, are more reliable from the standpoint of the normal metabolic picture (18). Since calcium phosphorylcholine chloride is relatively inactive pharmacologically (19), and is converted to inorganic phosphate after reaching the tissues, it may provide a valuable means for simultaneous administration of calcium and phosphorus in soluble form.

By expressing the specific activity of the phosphorus of the bone ash of the operated side as a percentage of that of the control side (table 3), data are obtained from which we may make an interesting observation. It is apparent in the case of the sectioned animals, that the humerus on the sectioned side has as high an isotope activity per milligram of phosphorus as that from the control side. This finding argues strongly for the concept that the phosphate of the bone salt is in rapid ionic equilibrium with blood phosphate (10, 20). If the radioactive phosphate appearing in bone was acquired solely by a process of accretion, these data should parallel the data on accretion following brachial section (table 2

<sup>3</sup> We have previously shown that the phosphorus from phosphorylcholine is liberated extremely rapidly, *in vivo*, as inorganic phosphate (1).

and fig. 2). This phenomenon of rapid ionic equilibration detracts greatly from the value of radioactive phosphate as a tool for the study of bone growth. It is conceivable, however, that this very situation may provide an indirect means of evaluating circulatory changes in bone, if it can be demonstrated that the level of exchange shortly after administration is related to the circulating blood volume of the bone.

#### SUMMARY

1. The daily parenteral administration of choline chloride had no significant effect on the course of denervation atrophy of the humerus or the flexor muscles of the forelimbs in the rat.

2. In our rats, weighing 150 to 250 grams, flexor muscles of the forelimb weighed 56 to 59 per cent less than their respective controls 24 days after denervation. However, no net loss of muscle mass occurred in the denervated limb after the time of operation. The apparent difference may be explained by normal growth in the control limb. Hypertrophic growth in the control limb was not apparent.

3. Following unilateral brachial section, the humerus continues to gain in mass after the time of operation, although this accretion is slower than normal. The unoperated control femur gave no evidence of hypertrophic growth.

4. The 24-hour uptake of radioactive phosphorus by bone ash is the same on an ash weight basis in normal and atrophic limbs. By far the greatest uptake must be by ionic exchange since the appearance of radioactive phosphorus in the femur does not parallel the accretion rate of the bone.

5. The phosphorus of trace-doses of calcium phosphorylcholine chloride does not enter bone ash preferentially to inorganic phosphate. Larger amounts of this compound might influence bone metabolism by saturating the system with calcium and phosphate.

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# A COMPARATIVE STUDY OF THE CHOLINESTERASE ACTIVITY OF THE VERTEBRATE NERVOUS SYSTEM, WITH ESPECIAL REFERENCE TO ITS RELATIONSHIP TO MOTOR ABILITY

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Recent investigations indicate that cholinesterase is present in considerable quantities in all nervous tissue. These findings have led to a number of studies attempting to discover the significance of this enzyme in the functional activity of the neurone. Most of the interest has centered around its possible rôle in the transmission of nerve impulses. In a recent study on the developmental behavior of amblystoma larvae, Sawyer (1943) presented evidence to support the concept that the cholinesterase content may be a biochemical criterion of the functional capacity in the neuromotor apparatus.

In order to test this concept through another approach, the present study deals with an analysis of the cholinesterase activity of the central nervous system of several representative vertebrates. An attempt is made to examine the relationship between the cholinesterase activity of the central nervous system and the general motor ability of the animal.

**MATERIAL AND METHODS.** The following animals, representative of three classes of vertebrates, were used in this study: Gold fish (*Carassius auratus*), Sucker (*Catostomus catostomus*), Shiner (*Notemigonus crysoleucas*), Frog (*Rana pipiens*), Necturus (*Necturus maculosus*), Tortoise (*Clemmys muhlenbergii*), Horned Lizard (*Phrynosoma*), and Giant Collared Lizard (*Crotaphytus collaris*). All the animals were obtained from commercial sources and were studied during the winter.

The central nervous system in each case is small enough to permit the use of the entire brain. However, only a part of the spinal cord was used. This consisted of a section 1.5 cm. long, directly posterior to the medulla.

The technique employed for counting the cell population was essentially the same as that described by Pearce and Gerard (1940) for frog brain tissue and by Lindeman (1943) for vertebrate retina. Briefly, the procedure is as follows: The brain and spinal cord were dissected from the animal and placed in Ringer's solution. All connective tissue and blood vessels were removed from the surface. The tissue was blotted carefully to remove surface moisture, and weighed. After weighing, the tissue was transferred to a small centrifuge tube containing 5 ml. of 25 per cent acetic acid saturated with carmine. Gentle shaking over a period of 30 minutes reduced the tissue to a fine suspension of intact nuclei and dissolved cytoplasm. Counts were made with a hemocytometer counting chamber. The nuclei of 25 large squares were counted for at least 6 samples. At least 3 suspensions were made for each species of animal studied. The number of nuclei per milligram of tissue was then calculated for each suspension. In an



attempt to obtain the relative nuclear area, the length and diameter of at least 300 nuclei were measured for each suspension. This was done with the aid of an ocular micrometer. Nuclei having the same dimensions were then grouped, and their individual as well as the total surface area computed. The total nuclear area per milligram of tissue was obtained by multiplying the mean nuclear area by the number of nuclei per milligram of tissue.

In computing surface area two formulae were employed. When the two measured diameters were not the same, the nucleus was assumed to be ellipsoid, and the following formula for surface area was used:

$$S.A. = \pi M \sqrt{\frac{M^2 F^2}{2}}$$

$M$  is the revolving, and  $F$  the fixed axis.

When the two diameters were equal, the mass was assumed to be a sphere and surface area was computed according to the formula:

$$S.A. = 4\pi r^2$$

The preparation of the material for the analysis of the cholinesterase activity was as follows: The brain and spinal cord were dissected from the animal and placed in Ringer's solution. After blotting and weighing, the tissue was transferred to a small test tube containing 0.5 ml. of glycine-NaOH buffer mixture and 500 mgm. of fine sterile white sand. The buffer mixture was prepared according to Tahmisian (1943), and had the following constituents: 90 cc. of molar/glycine, 10 cc. molar/NaOH, 9.94 grams of NaCl, 150 cc. of glycerine, the entire mixture made up to 1000 cc. with twice distilled water. The pH was found to be 8.4 with a Beckman glass electrode pH meter. The tissue was ground with a glass mortar until it formed a cell free suspension. The mixture was then further diluted with the glycine buffer solution until the final concentration of each sample contained the equivalent of 10 mgm. of tissue per milliliter of suspension.

The tubes were placed in a water bath kept constant at a temperature of  $27.5 \pm 0.02^\circ\text{C}$ . Preliminary experiments revealed that digestion is complete in less than two hours. After two full hours, the suspensions were removed from the bath, and centrifuged for two minutes. This left a clear milky solution which was decanted into another tube and constituted the enzyme extract.

The measurement of enzymatic hydrolysis was carried out as follows:

Sterile micro test tubes having a capacity of approximately 5 ml. were placed in metal racks and into each was pipetted exactly 1 ml. of freshly prepared 0.2 per cent acetylcholine chloride dissolved in glycine-NaOH buffer; 1 ml. of 0.1 per cent eserine-sulphate indicator (prepared according to Glick (1938)) was added to the tubes which were to serve as controls. (Generally, only one tube was used as a control for each three experimental tubes.) Exactly 0.5 ml. of the enzyme extract was then added to each tube and after thorough stirring, the tube was suspended in the water bath at  $27.5^\circ\text{C}$ . Hydrolysis was allowed to proceed at this temperature for one to two hours. At the end of this time,

hydrolysis was stopped in the experimental tubes by the addition of the eserine indicator solution. Colorimetric titrations were carried out with the bromthymol blue indicator and the end point checked each time with the Beckman glass electrode pH meter to insure an exact end point of 6.5. The titrations were made with 0.01N/HCl, using a micro burette graduated to 0.02 ml. The cholinesterase activity was computed in terms of the acid equivalent (ml. 0.01N/HCl) produced by enzymatic hydrolysis per milligram of tissue per hour.

**RESULTS AND DISCUSSION.** Among the studies upon the functional activity of the nervous system, the respiratory changes have been the most numerous. Most of the studies of that type are designed to measure resting metabolism and in no way indicate the true functional capacities of the tissue. Since there is a close parallelism between the electromotive force of action potentials and the cholinesterase content (Fulton and Nachmansohn, 1943) cholinesterase activity might logically be a measure of the functional potentialities of a tissue.

In an attempt to approach this problem, several animals were chosen from each of three classes of vertebrates. The basis for their selection depended upon voluntary locomotor activity, quickness of movement, mental alertness, and numerous behavior traits that characterize the animal as having either a sluggish or alert neuromotor response. For example, the turtle, necturus, and goldfish are considered to be relatively sluggish animals whereas the lizards, the frog and the shiner are quicker, more alert and active than their close relatives in the respective classes. With this in mind, a series of experiments was designed to measure the cholinesterase activity of the central nervous system in an attempt to establish any correlation between the enzymatic activity and relative neuromotor response.

The data contained in table 1 summarize the experiments on the central nervous systems of 8 different vertebrates. It is obvious that there is little justification for comparing the group as a whole. However, when the animals within each class are compared, it is interesting to note a close correlation between the cholinesterase activity and the relative motor ability. This is particularly evident among the amphibia and reptiles, where the necturus and turtle, two naturally sluggish animals, show a relatively low enzymatic activity in comparison to their more active relatives. The validity of this observation seems further justified when the cholinesterase activity is computed per cell in each class. This can be done by simply dividing the mean cholinesterase activity per milligram of tissue by the number of cells found in an equal mass. A summary of this data is found in table 1. Since these data are computed in terms of the mean, they represent values obtained upon hypothetical cells and do not take into consideration the volume or area of the cell. Since there is no adequate means available for computing the volume or area of a neurone, an alternative method has been employed for comparing the relative enzymatic activity of the cellular components within the central nervous system. The method employed involved the computation of relative surface area of the nuclei. This can be done by measuring the diameter of a representative sample of nuclei, and computing the mean. To obtain total nuclear area per milligram of tissue, the mean area is simply multiplied by the number of cells per milligram of tissue.

The data contained in the last column of table 1 form a summary of the cholinesterase activity when computed as a function of nuclear area. These data are only significant for purposes of comparison, if it can be assumed that at least a rough relationship exists between the total nuclear area and the total neurone area per unit of nerve tissue volume in all the animals studied. If one is justified in making such an assumption, then the cholinesterase activity per unit area of neurone should be proportional to the activity of this enzyme per unit area of nucleus. Since cholinesterase is largely concentrated at or near the surface of the nerve cell, (Boell and Nachmansohn, 1940) it would appear that the relative surface areas might logically provide a more accurate means of comparing its enzymatic activity. Thus, when one compares the cholinesterase activity of the various nervous systems, whether per cell, per unit of relative cell surface, or for a unit of the whole mass, it is of interest to note that there is a consistent

TABLE 1

*The cholinesterase activity of the central nervous system of several representative vertebrates in relation to motor ability*

ANIMAL	RELATIVE MOTOR ABILITY	CELLS PER MGM. TISSUE $\times 10^3$	MEAN S.A. OF THE NUCLEI $\times 10^{-4}$	TOTAL S.A. OF THE NUCLEI PER MGM. TISSUE	MEAN ChE* ACTIVITY PER MGM. TISSUE	STANDARD DEVIATION	ChE* ACTIVITY PER CELL $\times 10^{-7}$	ChE* ACTIVITY PER SQ. MM. S.A. OF NUCLEI $\times 10^{-3}$
Gold fish.....	Sluggish	600	0.958	57.48	0.0599	0.0011	0.99	1.042
Sucker.....	Active	500	1.160	58.00	0.0690	0.0007	1.38	1.200
Shiner.....	Very active	408	0.944	38.35	0.0710	0.0004	1.74	1.860
Necturus.....	Sluggish	34.2	18.630	63.66	0.0191	0.0010	0.55	0.300
Frog.....	Active	117	3.330	55.45	0.0476	0.00148	4.07	0.858
Tortoise.....	Sluggish	89.1	1.730	15.40	0.0282	0.00204	3.16	1.830
Horned lizard.....	Active	77.9	2.080	16.18	0.0537	0.00158	6.90	3.350
Collared lizard....	Very active	60.8	1.910	11.61	0.0650	0.00120	10.60	5.600

\* Cholinesterase activity represents the acid equivalent (0.01 N HCl) produced by enzymatic hydrolysis of acetylcholine per hr.

S.A. expresses surface area in square millimeters.

difference between the more sluggish animals and their more active relatives within the various classes. If a sufficient sampling has been used, and if one is justified in making the above comparisons, it would appear that a significant correlation does exist between the cholinesterase activity of the central nervous system and the motor ability of animals within a given class.

#### SUMMARY AND CONCLUSIONS

1. A quantitative study of the cholinesterase activity of the central nervous system was made upon 8 different animals representing 3 classes of vertebrates.
2. Animals were selected in each class which represented, as nearly as possible, extremes in general motor ability.
3. The enzymatic activity was determined by means of a microchemical method which involved the titration of the acid equivalent (ml. 0.01N/HCl) formed by the hydrolysis of acetylcholine.

4. When the cholinesterase activity was computed per cell, for a unit of the nuclear surface, and for a unit of the whole mass, a consistent correlation existed between the activity of the enzyme and the general motor ability of the animal within each of the classes.

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# STIMULATION OF LIVABILITY AND GLYCOLYSIS BY ADDITIONS OF GLUCOSE TO THE EGG YOLK-CITRATE DILUENT FOR EJACULATED BOVINE SEMEN

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A number of workers have shown that the semen of both man (3, 5, 9, 10) and the domestic animals (11, 14) contains glucose. Also, it has been shown that the amount of glucose decreased when the semen was incubated at body temperature (3, 5, 10, 11, 14). In some of these papers data were presented to show that this decrease in glucose was accompanied by an increase in lactic acid. However, little evidence was obtained to indicate that the break-down resulted in complete recovery of the glucose as lactic acid. From these observations it was concluded that spermatozoa obtained from glycolysis a certain proportion of their energy for motility.

According to Lardy and Phillips (6, 7) in the absence of glucose motility of bovine spermatozoa can continue at a maximum rate only by oxidation of certain phospholipids, or as a result of the oxidation of intracellular reserves. However, under anaerobic conditions the energy for motility is derived preferentially from glycolysis, the breakdown of glucose or other available carbohydrates to lactic acid. Presumably this type of metabolism would predominate in the usual test tubes employed for semen storage in routine artificial insemination. With human spermatozoa MacLeod (8) has indicated that, aerobically or anaerobically, the major source of energy for motility is derived from glycolysis.

Satisfactory diluters for the purpose of artificial insemination which maintain the fertility of properly handled bovine semen at high levels for a number of days have been developed by Phillips and Lardy (12), and by others (13). In fact, when bull semen is diluted with the egg yolk-phosphate or the yolk-citrate diluter and kept at 5°C., fertility may be maintained at an optimum level for 4 days (13, 17). However, improvement in diluters is still required to further increase the efficiency with which semen is used in practical artificial insemination of dairy cattle.

Studies in our laboratory have shown that bovine semen samples which reduced methylene blue in the shortest possible time, an indication of rapid glycolysis and usually an indication of great livability (16), sometimes failed satisfactorily to survive storage in stoppered test tubes at 5°C. in the yolk-citrate diluent. Some of such samples were found to be low in glucose when motility ceased.

It is the purpose of the present paper to report the results of a series of experiments designed to determine the effect upon spermatozoan livability during low-temperature storage of adding glucose to the yolk-citrate diluent. No similar study has been reported and, so far as the authors are aware, no similar

studies of spermatozoan metabolism have been made under long-time storage conditions at low temperature.

**EXPERIMENTAL.** The semen was collected and handled as previously described (18). On each fresh semen sample determinations were made of the spermatozoa concentration, initial percentage of progressively motile spermatozoa, and the methylene blue reduction time (2), all items considered important as indications of the quality of semen (16). Each ejaculate was then diluted with yolk-citrate or yolk-citrate-glucose at the rate of 1 part of semen to 4 parts of the diluent. Thus, one-half of each ejaculate was diluted with a yolk-citrate diluent, in which a small part of the citrate buffer had been replaced by an isotonic glucose solution. In the first experiment glucose was added at the rate of approximately 58 mgm. per 100 ml. of diluter, but the amount was doubled for the second and third experiments.

A 1 ml. portion of each diluted semen sample was then incubated for 1 hour at 46.5°C. as a preliminary estimate of its ability to survive storage at 5°C. (2). The remainder of each portion, about 7 to 8 ml., was stored in stoppered test tubes at 5°C. for 10 days after having been brought to the storage temperature in steps of a 5°C. drop each 20 minutes. Sub-samples were removed at the start and termination of incubation, before storage and at 2-day intervals for motility estimates and for chemical determinations on Somogyi (15) filtrates.

Glucose was determined by the Horvath and Knehr (4) adaptation of the Folin-Malmros micro-blood sugar method. Incubation of the diluters, with or without semen, with *Streptococcus fecalis* bacteria<sup>1</sup> indicated that about 20 to 30 mgm. per 100 ml. of the total reducing substances determined by this method in the diluters were non-fermentable. The total reducing substances are reported in this paper and are referred to as glucose. The Barker and Summerson (1) method was used for the determination of lactic acid.

The investigation was factorially designed for statistical analysis and three separate experiments were conducted at various times, nine, ten and eleven ejaculates being used in each experiment, respectively. There were seven separate observations or chemical analyses on each sample diluted with each diluter, or fourteen for each ejaculate. This fact made it possible to determine the influence of each ejaculate as well as the experimental treatment on the outcome of the investigations.

As the separate experiments were conducted at different seasons of the year with different bulls, the quality of semen as indicated primarily by methylene blue reduction time, spermatozoa count, and initial motility, varied markedly from experiment to experiment. The semen of experiment 1 was intermediate; while that of experiment 2 was poorest, and that of experiment 3 was best. The data on the average quality of the fresh semen used in each experiment are given in table 1.

**RESULTS.** The results of incubation for 1 hour at 46.5°C. including both motility observations and the data on glycolysis are shown in table 1 for each

<sup>1</sup> The authors are indebted to Prof. I. C. Gunsalus, Laboratory of Bacteriology, College of Agriculture, Cornell University for supplying us with these cultures.

individual experiment. Similar data for low-temperature storage are given in table 2, and are presented as means of the combined three experiments.

In each of the three experiments the addition of glucose to the egg-yolk citrate promoted increased motility, both at the end of incubation for 1 hour at 46.5°C. (table 1) and during low-temperature storage (table 2). Not only were more spermatozoa able to survive incubation and storage at the several time intervals, but the speed with which the individual spermatozoa moved, the

TABLE 1

*Average data concerning the semen used in each of the three experiments and results of incubation*

EXPERIMENT NUMBER	FRESH SEMEN				DILUTED SEMEN						
	No. of ejaculates	Spermatozoa/c.mm.	Initial motility		Diluter	MB reduction time	After incubation for 1 hour at 46.5°C.				
							Motility		Glucose loss	Lactic acid gain	Recovery
							%	rate			
		(1000's)	%	rate		min.			mgm./100 ml.	%	
1	9	1,327	63.3	3.7	YC	25.2	22.8	1.7	61.2	39.6	65
					YCG	25.2	29.6	1.8	54.5	40.2	74
2	10	880	58.0	2.6	YC	38.6	11.7	1.2	36.7	23.4	52
					YCG	38.7	17.6	1.5	39.7	25.6	59
3	11	976	74.5	3.8	YC	8.8	35.5	2.4	63.8	53.7	84
					YCG	8.2	41.8	2.7	70.7	57.1	81

YC = Yolk-citrate.

YCG = Yolk-citrate with added glucose.

TABLE 2

*Mean motility and glycolysis during storage at 5°C. for all experiments*

Days stored.....	DILUTER YC					DILUTER YCG				
	2	4	6	8	10	2	4	6	8	10
Motility %.....	44.0	32.1	23.1	15.9	12.4	49.3	39.0	29.4	22.0	16.4
Rate of motility.....	2.57	1.65	1.11	0.82	0.61	2.80	2.12	1.47	1.08	0.82
Glucose loss, mgm./100 ml.....	43.5	54.5	63.1	66.3	79.1	64.1	72.8	93.8	102.3	121.9
Lactic acid gain, mgm./100 ml.....	33.4	43.5	51.0	56.3	51.5	40.1	56.6	62.5	66.2	60.1
Recovery of glucose loss as lactic acid, per cent.....	76.9	79.7	80.9	84.8	65.1	62.6	77.8	66.7	64.8	49.3

rate of motility, was greater when glucose was added. These differences were highly significant mathematically. Also, the effect was noted for all semen samples, regardless of the actual level of glucose in the diluted semen either before or after storage and regardless of the concentration of spermatozoa in the semen.

As can be seen, the lactic acid production of the semen in the diluter to which glucose was added, was higher in every experiment than when no glucose was

added. This difference was statistically highly significant. The addition of glucose stimulated lactic acid production, as well as motility.

It is believed that this stimulation was due to the increased concentration of glucose in the diluent. In fact, in experiment 1, where an average of from 35 to 50 per cent more spermatozoa per cubic millimeter was found than in experiments 2 or 3, there was insufficient glucose added to some ejaculates for the large number of spermatozoa and they failed to live satisfactorily. It was for this reason that the amount of glucose was doubled in the second and third experiments. In no case in these investigations was the glucose level increased to the level found in fresh semen, which has averaged  $582.4 \pm 195.2$  ( $\pm$  standard deviation) mgm. per 100 ml. for 94 determinations on semen of varying quality. However, glucose additions to the diluter do not completely prevent the low livability sometimes observed in semen samples of extremely high concentration.

The decrease in the lactic acid levels found after 8 days' storage suggests that this acid was oxidized as the spermatozoa die off, or that this oxidation was a constant process which only became apparent when the rate of elaboration of

TABLE 3  
*Percentage of glucose loss recovered as lactic acid*

EXPERIMENT NUMBER	DILUTER USED									
	YC					YCG				
	2	4	6	8	10	2	4	6	8	10
Days stored										
1	77	78	85	79	59	63	68	55	51	23
2	41	44	39	52	36	31	45	37	36	27
3	104	123	123	118	103	89	118	107	101	91

lactic acid was decreased markedly. In experiments 1 and 2 the spermatozoa in a large proportion of the individual semen samples were dead at 8 days. In experiment 3, however, where some spermatozoa in every semen sample continued to live for from 16 to 22 days no decrease in lactic acid during the storage period of 10 days was observed.

One of the striking facts observed in this investigation was that, in spite of the greatly different qualities of semen used, the glucose loss was similar in each experiment. In fact, though the loss was greater in the portions of diluted semen to which glucose was added, the decrease after 10 days' storage was almost identical for the three experiments. The average glucose loss in the yolk-citrate at the end of the 10-day storage period was for experiment 1, 80 mgm. per 100 ml.; for experiment 2, 86 mgm. per 100 ml.; and for experiment 3, 72 mgm. per 100 ml. The loss in the yolk-citrate-glucose diluent was 123 mgm., 119 mgm. and 124 mgm. per 100 ml., respectively.

However, as can be seen in table 3, only in experiment 3, where the semen was of the highest average quality as measured by methylene blue reduction time, was nearly all of the glucose recovered as lactic acid. Also, only in this experi-



ment did the spermatozoa continue active, vigorous motility throughout the 10 days' storage. In fact, though the glucose continued to disappear after storage of 8 days in both experiments 1 and 2, 10 per cent or less of the spermatozoa were alive at this time, and the lactic acid content of the samples was decreasing. On the other hand, in experiment 3 some spermatozoa in each sample continued to live for from 16 to 22 days, and 35 per cent or more of the spermatozoa were actively motile after 8 days' storage.

In corroboration of data previously reported (16), a highly significant positive correlation was found between the total lactic acid gain during 10 days' storage and the percentage of motile spermatozoa at the end of the storage period (livability). Also, as earlier reported, no relation was shown between the glucose loss and livability. The correlation coefficients found were as follows:

	<i>Correlation coefficients between livability and:</i>	
	<i>Glucose loss in 10 days</i>	<i>Lactic acid gain in 10 days</i>
In yolk-citrate diluent.....	0.07	0.55
In yolk-citrate-glucose diluent.....	0.13	0.73

Only the coefficients between lactic acid gain and livability are of a magnitude to be considered mathematically significant in terms of the number of observations made (30 paired observations). These observations support the viewpoint that energy of glucose break-down supports motility and life of spermatozoa during low-temperature storage only if the glucose is glycolyzed to lactic acid.

Also, the data suggest that, if the glucose was not glycolyzed by the spermatozoa, it was oxidized by systems introduced by the semen, for the rate of glucose disappearance in each of the three experiments was entirely similar, though the level of lactic acid gain was markedly different. That the glucose loss was due to systems introduced by the semen is indicated further by the fact that neither of the diluters stored alone changed in either glucose levels or lactic acid content throughout a 10-day storage period at 5°C.

Obviously, the statement that glucose was oxidized has not been proven by this investigation for the methods used could not answer that question. However, this interpretation is indicated by the data, and will be the subject of future investigations.

The variation in glucose loss recovered as lactic acid for the three experiments indicates that the inherent quality of the semen used is an important item to be considered in any study of semen physiology. Apparently glucose may be used by semen samples in the presence of yolk-citrate by either glycolytic or respiratory systems, but only glycolysis results in the production of energy which may be utilized by the spermatozoa for motility.

With the best semen all of the glucose used during storage was transformed to lactic acid. With samples of lower inherent quality a variable quantity of the glucose was glycolyzed to lactic acid, depending upon the number of spermatozoa present and their ability to live. In the case of experiment 3 apparently other substrates than glucose were converted to lactic acid. During low-temperature storage, especially where no glucose was added, more lactic acid was recovered than glucose was lost.

## SUMMARY

In an investigation to determine the effect of adding from 58 to 116 mgm. of glucose per 100 ml. to bovine semen diluted at the rate of 1 part of semen to 4 parts of the yolk-citrate diluent and incubated for 1 hour at 46.5°C. or stored for 10 days at 5°C., it was found that:

1. The added glucose promoted increased livability and lactic acid production during incubation for 1 hour at 46.5°C., and during storage for 10 days at 5°C.

2. The stimulation of motility duration and lactic acid production occurred in spite of the fact that the initial glucose stores of the diluted semen samples were not depleted.

3. The glucose loss during low-temperature storage in the three separate experiments proceeded at similar rates regardless of the quality of semen used.

4. Glucose loss was not directly related to the livability of the spermatozoa unless that sugar was glycolized to lactic acid.

5. The proportion of glucose loss recovered as lactic acid was dependent upon storage interval and upon the quality of the semen used in the separate experiments and varied from less than 25 per cent to complete recovery.

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# THE EFFECTS OF BLOOD FLOW AND ANOXIA ON SPINAL CARDIOVASCULAR CENTERS

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The well known studies of Heymans have established the importance of the carotid and aortic pressure receptors and chemoreceptors in the buffer regulation of the cardiovascular system. There is considerable evidence, however, which demonstrates the existence of subsidiary factors which also play a rôle in this type of regulation. In the spinal animal, in which the action of the buffer nerves is excluded, there are cardiovascular adjustments to both pressure and chemical changes. In regard to chemical changes, Kaya and Starling (9) and Mathison (13) demonstrated that anoxia produces a compensatory rise in blood pressure in the spinal animal. These authors attributed this response to direct action of anoxia on the spinal centers, but they did not take into consideration the possibility of reflexes initiated by peripheral chemoreceptors. Concerning responses to pressure changes, Gammon and Bronk (5) have presented evidence which would indicate that the mesenteric Pacinian corpuscles are stimulated by changes in the calibre of the intestinal blood vessels, and therefore are likely sources of afferent impulses for spinal cardiovascular reflexes. Using the cross perfusion technique on the spinal animal, Heymans and co-workers (8) obtained compensatory responses to changes in blood pressure which they interpreted as reflexes initiated by these Pacinian corpuscles. Their data fail to establish the reflex nature of the response, however, and, as they recognize, their results would demand a different response of the Pacinian corpuscles than that which had been observed by Gammon and Bronk. The fact that similar vascular responses may be observed in the absence of reflexes has been demonstrated by Brooks (4) who studied the vascular responses of spinal cats in which all dorsal roots had been sectioned. These deafferent animals exhibited compensatory elevations in blood pressure following a sudden hemorrhage. Since leg volume measurements gave evidence of vasoconstriction and since other functions of the sympathetic nervous system showed parallel increases in activity after hemorrhage, Brooks concluded that the drop in blood pressure produced by hemorrhage acts directly on the autonomic centers in the spinal cord to increase vasomotor tone. Whether this action was due to the fall in spinal blood pressure itself, or whether it was an asphyxial effect resulting from inadequate circulation to the cord, was not established.

The combined evidence of these and other investigators makes it clear that there are subsidiary buffer mechanisms operating at the spinal level, but in no case has the exact mechanism of the reaction been conclusively established. In attempting to clarify this problem, it seemed of value to employ the technique of direct nerve recording, since in this manner all uncertainty as to the neurogenic

nature of the response may be eliminated. The work of Bronk and his associates (2, 14) has shown the inferior cardiac nerve of the cat to be an ideal preparation for such a study. This nerve exhibits a considerable degree of tonic activity under normal experimental conditions—activity which is rather dramatically inhibited by a rise in blood pressure. The fact that this activity shows synchrony with the pulse pressure and that changes in this activity correlate very closely with expected cardio-acceleratory and vasomotor readjustments appears to justify its use as an index of the activity in the sympathetic outflow to the cardiovascular system as a whole. By employing this direct measure of sympathetic activity, it has been possible to demonstrate conclusively that changes in oxygen tension act directly on the spinal cardiovascular centers to alter their activity, and that changes in blood pressure with resultant changes in blood flow are capable of altering this activity by virtue of the fact that they alter the oxygen supply to these centers.

**METHODS.** The results reported here were all obtained from acute experiments performed on cats lightly anesthetized with chloralose. For recording the activity in the inferior cardiac nerve, a section of the ventral chest wall including the first four or five ribs was removed, the animal being maintained with artificial respiration. The inferior cardiac nerve (or one of its branches) was sectioned at the point where it passes over the aortic arch and dissected back to within about 1 cm. of the stellate ganglion. The potentials obtained from the free end of this nerve were amplified with conventional capacity coupled amplifiers and recorded by means of a General Electric mirror oscillograph in conjunction with a bromide paper camera.

In all operative procedures, great care was taken to assure the completeness of the various sections carried out. In the case of the carotid sinus, for example, a distinct sinus nerve may usually be identified and sectioned, but this procedure in itself did not give assurance of the complete elimination of all possible afferent impulses from the sinus. Therefore, after preliminary exposure and denervation of the sinus, the common carotid was ligated. By placing caudal traction on this ligature, it was possible to place a hemostat obliquely across the internal and external carotids and their branches about 5 mm. above the carotid sinus and glomus. Finally, the isolated sinus was cut open, blood expelled from it, and observed for several minutes to make certain that there was no residual bleeding. This procedure eliminated the possibility of collateral circulation exerting back pressure on the sinus and thereby stimulating any afferent fibres which had escaped section. Dorsal root and cord sections were performed intradurally after suitable laminectomy. In cases of any possible uncertainty as to the success of the operative procedures, the animal was carefully autopsied at the conclusion of the experiment. This was done routinely in cases of dorsal root section.

A cannula inserted into the saphenous vein was used for injections, and a femoral or carotid artery used for blood pressure recording. The latter was recorded optically using a dampened membrane manometer. A Starling type "Ideal" respirator was employed for maintaining artificial respiration. Gas mixtures contained in Douglas bags could be admitted to the respirator as desired by the use of appropriate valves.

**RESULTS.** The tonic activity found in the inferior cardiac nerve after the carotid and aortic nerves have been eliminated and the effect on this activity produced by a rise in blood pressure is shown in figure 1A. In the animal with buffer nerves intact, the tonic activity is typically restricted to barrages of impulses in synchrony with each pulse wave, suggesting that the tonic activity

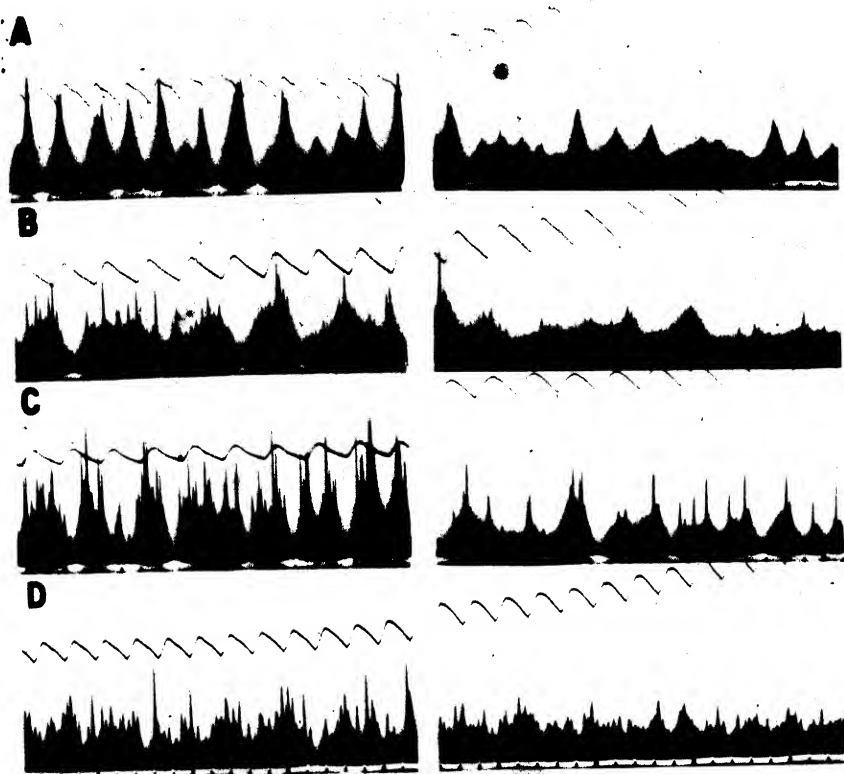


Fig. 1. Inhibition of tonic activity in inferior cardiac nerve after removal of afferents. Base line in all figures gives time intervals of 1/5th seconds.

A. Carotid and aortic buffer nerves eliminated. Adrenalin (1 cc. 1:50,000) injected during gap in record. Note synchrony of activity with pulse in initial portion and inhibition of activity as blood pressure rises.

B. Same animal as above shortly after cord and sympathetic chain sections at  $T_6$ . Higher amplification than in A. Blood pressure raised with adrenalin.

C. Similar preparation with buffer afferents removed and cord and sympathetic chain sections at  $T_6$ . At gap in record, aorta occluded at level of diaphragm.

D. Deafferent preparation; cord sections at  $C_6$  and  $T_6$ . Adrenalin injection.

is periodically inhibited by impulses initiated in the buffer afferents with each systolic peak of pressure. Evidence supporting this assumption is found in the fact that when these buffer afferents are eliminated, the tonic activity becomes largely random and according to others (14) no longer shows synchrony with the pulse pressure. In our experience, however, some synchrony with the pulse pressure still persists, as is evident in the initial portion of figure 1A. The very

persistence of this pulse synchrony suggests the presence of some additional mechanism tending to inhibit activity with each pulse wave. Furthermore, even though the buffer nerves have been eliminated, a rise in blood pressure produces a definite reduction in the tonic sympathetic activity, as shown in the latter portion of figure 1A. This response is in contrast to that in the normal animal where a comparable rise in blood pressure leads to a complete inhibition of activity; nevertheless an incomplete but definite reduction in activity similar to that shown here was obtained in repeated experiments.

In order to eliminate impulses from mesenteric receptors, the splanchnic nerves were then sectioned. Whether this had any minor effect on the activity in the inferior cardiac nerve could not be accurately determined; grossly the same type of responses was obtained as that shown in figure 1A. To totally exclude all possible afferent impulses from the caudal half of the animal, the spinal cord and the sympathetic chains (including visceral afferents) were sectioned at a mid-thoracic level. As was to be expected, acute section of the thoracic cord greatly depressed the animal, but with sufficient amplification considerable residual activity could be recorded in the inferior cardiac nerve. Such an animal, lacking connection with all receptors below the thorax and with aortic and carotid receptors eliminated, still gave definite evidence of a reduction in tonic sympathetic activity when the blood pressure was raised with adrenalin (fig. 1B).

The use of adrenalin to elicit such rises in blood pressure was a natural selection, but it introduces one possible complication in that adrenalin in sufficient concentration has a direct inhibitory action on transmission in sympathetic ganglia (12). To avoid this complication, blood pressure was raised by pitressin injections, and reductions in the tonic sympathetic activity were obtained which were quite comparable with those obtained with adrenalin. In order to eliminate pharmacological complications completely, moreover, thoracic blood pressure was raised by suddenly occluding the aorta at the level of the diaphragm. This procedure served the additional purpose of restricting the possible locus of the blood pressure effect, since the rise in pressure in the anterior half of the animal was at the expense of a fall in blood pressure in the caudal half of the animal. The results obtained by this sudden occlusion of the abdominal aorta were typical of that illustrated in figure 1C where inhibition of tonic activity with a rise in blood pressure is again evident. This inhibition could not have been due to afferent impulses entering the spinal cord over thoracic dorsal roots, since it was found to be unaffected by sectioning all dorsal roots above the level of cord transection.

In view of these preliminary results, it appeared highly improbable that the persisting inhibitory response was of reflex origin. To demonstrate this point conclusively, it was necessary to study the spinal cord and its motor outflow isolated from all other nerve influences. This was accomplished by combining low cervical and mid-thoracic transections of the spinal cord, section of all dorsal roots between levels of cord transection, and bilateral section of the thoracic sympathetic chains above and below the ganglia corresponding to the lower level of cord section. This isolated the upper thoracic cord (representing the

spinal outflow to the inferior cardiac nerve) from all other nerve connections including its own dorsal roots. To eliminate any complications arising from vagal influence on the heart, the vago-sympathetic trunks were also sectioned bilaterally. This type of preparation was used in all of the remaining experiments and will be referred to as the "deafferent spinal" preparation. Somewhat contrary to expectations, tonic activity could still be recorded in the inferior cardiac nerve in spite of the severe state of depression following acute section of the cervical cord. Furthermore, the deafferent spinal preparation was found to exhibit a definite reduction in this residual tonic activity when the blood pressure was raised by adrenalin (fig. 1D) or by sudden occlusion of the aorta at the level of the diaphragm (fig. 2A).

During the initial stages of this investigation, pressor drugs had been employed in the hope of clarifying the possible rôle which might be played by hitherto unrecognized pressure receptors. Various observations, however, pointed to the fact that the inhibitions seen in records such as 1C and 2A were due to changes in blood flow rather than to changes in blood pressure *per se*. One indication of this was that release of the clamp occluding the aorta with a consequent sharp drop in thoracic blood pressure was not accompanied by any immediate resumption of tonic activity, as would be expected if the inhibitory effect was purely one of pressure. On the contrary, it was a matter of a minute or more before the tonic activity returned to the control level, suggesting that the increase in blood flow had produced some change in the tissues of the spinal cord which persisted for a short time after blood flow had been reduced to the original levels. The most obvious factor which might account for this relationship between tonic activity and blood flow would be alterations in blood gas equilibria within the tissues of the cord. In support of this assumption was the fact that changes in the rate of artificial respiration had marked effects in altering the amount of tonic activity recorded in the nerve.

Records from an experiment illustrating these effects of changes in ventilation are shown in figures 2B-D. After completing the occlusion experiment recorded in figure 2A, the clamp was removed from the aorta and the respirator speeded up so as to hyperventilate the animal. The change produced by this hyperventilation is shown in figure 2B. By comparing the activity in this record with that in the initial portion of figure 2A, a definite reduction in tonic activity may be seen. The respirator was then stopped, and shortly thereafter a progressive increase in sympathetic activity was noted. During the course of this asphyxial rise in cardio-accelerator tone, the aorta was again clamped, and in figure 2C it will be seen that the rise in blood pressure produced by this occlusion failed to inhibit the nerve activity. When, however, the respirator was turned on with the clamp still occluding the aorta, the activity fairly shortly subsided to the original level (fig. 2D). Since the blood pressure rise failed to show any inhibitory effect in the absence of adequate aeration of the blood, it is apparent that the inhibitory response previously obtained is not a direct effect of pressure, but rather the result of an increase in the flow of normally aerated blood to the spinal cord.

There remains the problem as to whether this effect is due to alterations in the supply of oxygen to the tissues or to alterations in the removal of carbon dioxide from the tissues. To settle this point, the effects of hypercapnia and anoxia were tested on well ventilated preparations by the introduction of various gas mixtures into the respirator. The acute depression of the circulatory system in these deafferent spinal preparations, however, made it difficult to carry the

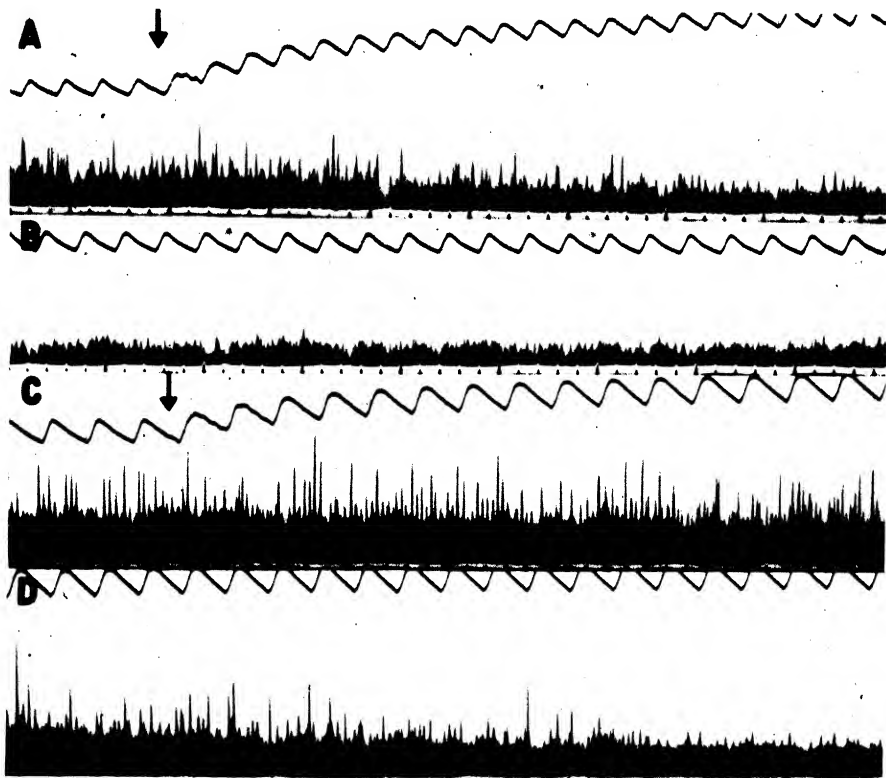


Fig. 2. Effects of respiration on tonic activity and blood pressure response. All records obtained with the same amplification from the same deafferent spinal preparation; cord sections at C<sub>6</sub> and T<sub>6</sub>.

A. Animal ventilated at normal rate; aorta occluded at arrow.

B. Activity after one minute of hyperventilation.

C. Activity after one minute of asphyxiation; aorta occluded at arrow. Note absence of inhibition.

D. Clamp still occluding aorta; activity falls as respiration is resumed.

animal through repeated exposures to anoxia. In several experiments, therefore, the axial and carotid arteries, the abdominal aorta, and the corresponding veins were ligated, thereby restricting the cardiac output to the thoracic aorta and its branches. This procedure served to maintain thoracic blood pressure at adequate levels for a series of experiments. In addition, although there remained some collateral circulation to other parts of the body,



the immediate effect of the experimental alterations in blood gas tensions would be largely restricted to the vascular bed supplied by the intercostal arteries, in which the thoracic cord is the essential significant structure.

Recordings from such an experiment performed on a deafferent spinal animal with restricted circulation are shown in figure 3. The first record (3A) shows

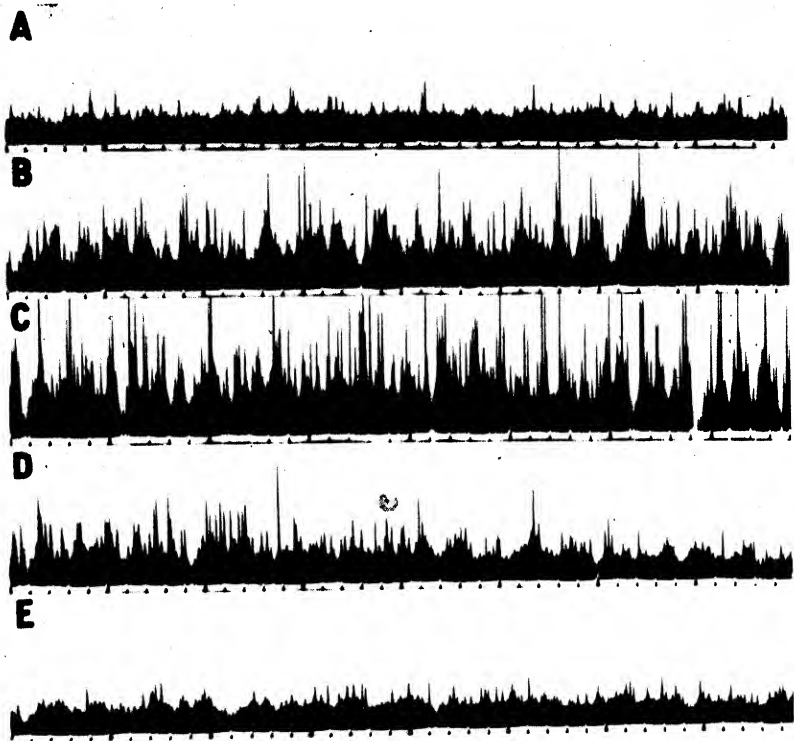


Fig. 3. Effects of anoxia. All records with same amplification and from same spinal deafferent preparation; cord sections at  $C_6$  and  $T_6$ . Circulation restricted as described in text.

A. Ventilation at moderate rate with room air.

B. Activity after 90 seconds' ventilation with nitrogen.

C. Further increase in activity with anoxia, five seconds of recording omitted between B and C.

D. A mixture of 90 per cent  $O_2$  and 10 per cent  $CO_2$  substituted for the nitrogen. Record shows decrease in activity as oxygen reaches tissues.

E. Return to original low level while being ventilated with  $O_2$ - $CO_2$  mixture; 5 seconds of record omitted between D and E.

the activity present in the inferior cardiac nerve when the animal was being ventilated at a moderate rate with room air. A valve was then turned so as to feed the inlet of the respirator with pure nitrogen. After a latency of about 40 seconds (20 sec. of which was attributable to dead space) an increase in nerve activity could be detected. Within 90 seconds, this increase had become very pronounced (figs. 3B and 3C). A mixture of 90 per cent oxygen and 10 per cent carbon

dioxide was then substituted for the nitrogen. When oxygen again reached the tissues, the activity rapidly lowered (fig. 3D) and returned to the initial low level (3E). This demonstration of a marked increase in activity when the animal is ventilated with nitrogen free of carbon dioxide and its inhibition by ventilating with oxygen containing 10 per cent carbon dioxide makes it clear that the stimulatory effect of asphyxiation seen in figure 2 is due to anoxia and can not be attributed to tissue hypercapnia.

The blood pressure responses in the deafferent spinal animal accompanying the anoxia were somewhat variable, but in general were in agreement with the responses which have been previously reported for the effect of anoxia on the spinal animal (13). In the normal animal, reflex and central stimulatory effects appear to predominate initially over the developing anoxic failure of the heart, and there is a corresponding initial rise in blood pressure. In the spinal animal, however, the remaining compensatory mechanisms are not able to completely correct for anoxic failure of the heart muscle, and as a consequence there tends to be a progressive fall in blood pressure with little or no indication of an initial rise. That this drop in blood pressure is due to heart failure was evidenced in these experiments by gross ventricular dilatation, development of heart block, and frequently the appearance of fibrillation if the anoxic period were not fairly shortly terminated. That this drop in blood pressure did not relate to diminished sympathetic tone was evidenced by the greatly increased activity seen in the inferior cardiac nerve (fig. 3C), and also by a very dramatic rise in blood pressure immediately after the anoxic period, which would appear to represent recovery of the heart muscle before the oxygen tension in the spinal centers had been adequately restored. It should be emphasized, moreover, that the increase in nerve activity is in no way dependent upon the cardiac failure and consequent drop in blood pressure. In many experiments the activity of the inferior cardiac nerve showed a definite increase before the blood pressure had fallen more than 10 mm. If the circulation to the abdominal viscera was not occluded, it was possible in a few cases to obtain an initial rise in blood pressure with the anoxia. It is quite possible that liberation of adrenalin by the sympatho-adrenal system might have contributed considerably to this rise in blood pressure (10). However, the fact that a marked increase in tonic activity still occurred in the cases where the blood pressure rose indicates that the stimulatory action of anoxia is not dependent upon a failing circulation and consequent capillary stasis within the spinal cord.

Since sympathetic ganglia are interposed between the cord and the postganglionic fibres from which the activity was recorded, the question arises as to whether this stimulatory effect of anoxia is necessarily acting on the spinal cord. In this connection, it should be pointed out that the anoxic stimulation was purely a transient phenomenon. Within about a minute of the time that this activity became marked, there was an abrupt disappearance of all activity from the nerve. This stage of failure was reversible if oxygen was restored fairly promptly. In contrast to this picture, Bronk and Larrabee (3) working with the same nerve preparation as that employed here, found that occlusion of the blood

supply to the stellate ganglion with consequent stasis and anoxia did not block ganglionic transmission until periods of well over 30 minutes. Somewhat similar results were obtained by Bargeton (1) who studied the superior cervical ganglion. In neither study was there any report of activity arising spontaneously within the ischemic ganglia; in fact there is considerable evidence which indicates that sympathetic ganglia are incapable of independent spontaneous activity (7). If the preganglionic fibres were subjected to considerable stimulation during the ischemic period, an initial period of facilitation of transmission was observed. However there is no basis for attributing this facilitation to anoxia; Bargeton favored the view that it was caused by accumulation of excess potassium. These studies of the ganglia, therefore, do not offer any explanation for the picture which has been observed here. Furthermore, in experiments such as that shown in figure 3, it was found that destruction of the thoracic cord completely abolished the activity in the inferior cardiac nerve of the surviving preparation regardless of the gases with which it was ventilated. It therefore appears justifiable to exclude anoxic effects on the ganglia from any significant contribution to the responses observed here, and regard these changes in tonic activity as being due primarily to a direct action of anoxia and blood flow on the cardiovascular centers within the spinal cord.

**DISCUSSION.** This study constitutes a direct demonstration of the fact that alterations in cardio-accelerator tone may be produced in the deafferent spinal animal by alterations in blood flow to the spinal cord. Assuming that these changes in cardio-accelerator tone are paralleled by changes in vasomotor tone, the evidence serves to confirm the similar conclusions of Brooks in his interpretation of the compensatory changes in blood pressure following acute hemorrhage. The data further indicate that the significant factor in blood flow is the resultant alteration in oxygen supply. In evaluating these findings, the acute nature of the experiments reported here should be emphasized. There has been a tendency by some to regard the compensatory changes seen in the chronic spinal animal as the development of mechanisms after cord section which were dormant and nonfunctional in the normal animal. This can not apply to the experimental results reported here, since in some cases recordings were made within ten minutes of the time of cord section, at which time the compensatory response to anoxia was demonstrable.

The significance which these findings have for the compensatory "reflex" effects in the spinal animal reported by Heymans and co-workers (8) (cf. also Simister and Conklin (15)) is problematical. It appears quite possible that these supposed reflex responses might have been due to changes in blood flow to the spinal centers, involving the same mechanism as that studied here. On the other hand, the present data in no way exclude the possibility that the Pacinian corpuscles or other as yet unknown pressure or chemoreceptors may play a significant subsidiary rôle in cardiovascular regulation. Any future investigation designed to demonstrate the action of such receptors, however, must take due precaution to differentiate reflex mechanisms from the direct action of blood flow and anoxia on the spinal cardiovascular centers.

This demonstration of a stimulatory action of anoxia on spinal cardiovascular centers necessitates a modification of the view which has been held by Gellhorn and his associates that anoxic stimulation of the cardiovascular system is due exclusively to reflexes initiated by stimulation of the peripheral chemoreceptors (11, 6). As a matter of fact, in the more recent of these two studies (6) it is reported that other spinal autonomic centers are stimulated by anoxia, but no data are given on the cardiovascular response to anoxia in the spinal animal.

The finding of tonic activity in the acute deafferent spinal preparation was somewhat unexpected and is in itself an observation of considerable interest. Since observations have not been extended to the chronic preparation, the possibility that this activity was dependent on irritative effects from trauma of the cord can not be excluded. Arguing against this possibility is the fact that in a total of 10 deafferent spinal preparations which have been studied, tonic activity was uniformly present and control recordings showed no significant variation over periods of four to five hours during which observations were made. Furthermore, if the tonic activity is to be explained on irritative grounds, then the increase in activity with anoxia must be considered as facilitation of these irritative stimuli. The magnitude of this anoxic increase in activity, especially when related to the negligible amount of activity found in the hyperventilated animal, makes any such explanation seem highly improbable.

It would be of interest to know how much of a rôle the oxygen tension in the spinal cardiovascular centers may contribute to cardiovascular tone in the normal animal. Although the reaction to anoxia reported in these experiments is rather marked, the experimental procedures were such that the entire circulatory system was too acutely depressed to be in any way comparable with the normal condition. Hence any direct translation of these results to responses of the normal animal is quite unjustified. Our present knowledge of the mechanisms involved in cardiovascular regulation would indicate that the buffer reflexes are dominant to any action of oxygen at spinal centers, and doubtlessly they would intervene to correct circulatory disbalances before the spinal centers could become markedly anoxic. The very fact that anoxic stimulation of the spinal centers could be demonstrated in spite of the extreme depression of the acute preparations, however, suggests that oxygen tension may play some rôle in determining the general excitatory state of the spinal centers in the normal animal, thereby serving to reinforce the buffer reflex mechanisms. It is obvious that this action of anoxia on spinal centers would assume a more important rôle in states of acute depression such as those seen in neural or circulatory shock, and also serve as a safeguard to protect the spinal cord from any circulatory inadequacies which might result from local interference with its blood supply.

*Acknowledgment.* I wish to express my appreciation to Dr. Robert F. Pitts, upon whose suggestion this investigation was undertaken, for his many valuable suggestions and constructive criticisms.

## SUMMARY

By employing the activity in the inferior cardiac nerve of the cat as a direct index of the activity in the sympathetic outflow to the cardiovascular system, the following conclusions have been reached:

1. After removal of all possible sources of reflex inhibition, a rise in thoracic blood pressure produced by injections of adrenalin or pitressin or by sudden occlusion of the abdominal aorta still serves to inhibit the tonic activity in the thoracic sympathetic outflow to the cardiovascular system.

2. The tonic activity arising within the spinal cardiovascular centers of deafferent spinal preparations is sensitive to changes in oxygen tension. This activity is depressed by hyperventilation or ventilation with a mixture of 90 per cent  $O_2$  and 10 per cent  $CO_2$ , and stimulated by asphyxiation or ventilation with pure nitrogen.

3. In the deafferent spinal preparation, a rise in blood pressure inhibits sympathetic activity by increasing the flow of blood to the spinal cord. This increases the supply of oxygen to the spinal cardiovascular centers and thus depresses their activity.

4. It is suggested that the oxygen tension in the spinal cord of the normal animal may contribute to the excitatory state of the spinal cardiovascular centers, and thereby reinforce the buffer reflexes which are integrated at higher levels of the nervous system.

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# DETERMINATION OF CARDIAC OUTPUT IN THE DOG BY THE FICK PROCEDURE

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Early use of the Fick procedure for determination of cardiac output in the dog (1) and the horse (2) involved the drawing of venous blood from the "right heart" (1) or the right ventricle (2). Recent applicants of the procedure to man (3, 4) and the dog (5, 6, 7) have taken venous blood from the right auricle. With this technique, we have seen large variations in cardiac output in repeated determinations in the dog (8). Examination of the results of three such experiments (table 2, expts. 1-3) suggested that the variation did not depend on changes in oxygen consumption or arterial oxygen content, but on changes in venous oxygen content. Two likely explanations, (1) failure to obtain a representative sample of mixed venous blood, (2) change in cardiac output with time, have been investigated.

**EXPERIMENTS.** Dogs were anesthetized with ether long enough to permit the intravenous injection of 250 mgm. per kgm. of sodium barbital. A tracheal cannula was inserted, and connected with a spirometer of the Benedict-Roth type, or with a flutter-valve system to separate expired air, which was collected in a Douglas bag. Oxygen consumption was measured from the spirometer tracing or by determination of the volume and composition of the expired air, which was analyzed with a 25 cc. Orsat-Henderson apparatus. Respiratory minute volume and CO<sub>2</sub> production were calculated from these data. Blood was collected from various parts of the circulation by cannula or needle puncture without exposure to air, over mercury (9). Duplicate analyses for oxygen on 2 cc. samples or oxygen and carbon dioxide on 1 cc. samples were done by the manometric method of Van Slyke and Neill (10). Cannulae were introduced into the venae cavae and the right auricle by way of the right external jugular vein. Steel cannulae were used, the apertures being 70 mm. apart for the venae cavae, 25 mm. apart for two points in the auricle. Blood from the right ventricle was obtained by needle puncture (11). The location of the cannulae and the site of needle puncture were determined *post mortem*. Mean arterial pressure was recorded by a Hg manometer, and pulse rate counted from its tracing.

**RESULTS.** The precision of the blood gas analysis is indicated by the average difference of duplicate oxygen analyses, which was 0.096 cc. per 100 cc. for 89 pairs. In addition, blood was drawn simultaneously, by means of a cannula and a needle from a well-mixed sample over mercury, and analyzed for oxygen.

<sup>1</sup> A preliminary communication of some of these data appeared in *Federation Proceedings* 3, March, 1944.

In two such experiments, the average difference in oxygen content was 0.175 cc. per 100 cc.

The results of simultaneous sampling of venous blood at different points are shown in table 1. In four pairs of samples from the venae cavae, the oxygen

TABLE 1  
*Oxygen content of simultaneously drawn samples of venous blood*

Oxygen content of simultaneously drawn samples of venous blood											
EXP. NO.	SIMULTANEOUS* SAMPLES	OXYGEN CONTENT CC. PER 100 CC. BLOOD						OXYGEN CON-SUMP-TION	CARDIAC OUTPUT	ARTE-RIAL PRES-SURE	
		Vena cava		Right auricle			Right ven-tericle				Fe-moral artery
		Sup.	Inf.	Ceph.	Caud.	Center					
1	Auricle			18.14	18.15			21.20	59.7	1.96	118
2	Auricle			15.61	15.83						116
3	Auricle venae cavae	14.25	16.08	15.04	17.22						
4	Auricle venae cavae	16.96	19.96	21.26	22.08						120 110
5	Auricle, vena cava	19.55			19.36			21.30	126	6.51- 7.32	145
6	Auricle, ven- tricle					10.37 12.32 7.05	14.82 14.89 14.00				127 123 114
7	Auricle, ven- tricle			10.37	11.64	16.70 16.41	15.83 15.83 11.36				164 145 92
8	Auricle, ven- tricle			12.82	13.40	10.67 10.66	13.93 10.65 13.57				137 92 114
9	Auricle, ven- tricle					21.95	21.27	25.39	102	4.39- 6.22	128
10	Vena cava, ventricle	14.89					15.43				100
11	Venae cavae, ventricle	12.16	13.56				12.95	16.85	122	3.12	135

\* Figures for oxygen content on the same line were simultaneous samples.

content was always greater in the inferior vena cava, with an average difference of 2.57 cc. per 100 cc. In six pairs of samples from two points in the right auricle, the average difference in oxygen content was 0.86 cc. per 100 cc. In twelve pairs of samples from the right auricle and right ventricle, the average difference was

1.79 cc. per 100 cc. In one experiment, not shown in the table, in which the oxygen content of superior and inferior vena caval blood differed by 4.04 cc.

TABLE 2  
*Serial determinations of cardiac output*

EXP. NO.	TIME*	METHOD†	RESPIRATORY VOLUME l per min.	OXYGEN CONSUMPTION cc. per min.	CO <sub>2</sub> PRODUCTION cc. per min.	R.Q.	OXYGEN CONTENT		CO <sub>2</sub> CONTENT		A-V DIFFERENCE		CARDIAC OUTPUT		ARTERIAL PRESSURE mm. Hg	HEART RATE PER MIN
							Arterial	Venous	Arterial	Venous	O <sub>2</sub>	CO <sub>2</sub>	O <sub>2</sub>	CO <sub>2</sub>		
	min.						cc. per 100/ cc.		cc. per 100/ cc.		cc. per 100/ cc.		l. per min.			
1‡	x	Closed		89			21.63	18.64			2.99		2.97		190	147
	x + 20	Closed		92			22.64	16.03			6.61		1.39		162	144
	x + 60	Closed		90			22.47	15.78			6.69		1.34		168	135
	x + 240	Closed		111			23.17	15.73			7.44		1.49		152	159
2‡	x	Closed		128			25.97	23.15			2.82		4.54		156	180
	x + 20	Closed		121			25.91	21.37			4.54		2.66		148	144
3‡	x	Closed		133			21.85	19.03			2.82		4.72		171	168
	x + 20	Closed		138			21.31	17.90			3.41		4.05		166	180
4	180	Closed		152			19.87	14.95			4.92		3.09		133	
	210	Closed		129			20.19	13.58			6.61		1.96		121	
5	103	Closed		176			21.41	17.51			3.90		4.50		151	186
	154	Closed		157			18.03	12.03			6.00		2.62		116	180
	175	Closed		143			17.70	10.98			6.72		2.12		87	174
6	140	Closed		170			23.98	18.52			5.46		3.11		157	174
	173	Closed		173			23.43	17.31			6.13		2.83		140	174
7	68	Closed		116			15.61	13.05			2.56		4.53		134	
	101	Closed		110			15.32	11.80			3.52		3.13		122	
8	105	Open	4.50	96	44	0.46	22.02	18.40	45.51	48.35	3.62	2.84	2.83	1.55	135	164
	195	Open	4.24	99	59	0.60	21.79	16.48	45.78	49.97	5.31	4.19	1.86	1.41	153	171
	274	Open	3.75	110	70	0.64	20.04	14.37	49.87	52.24	5.67	2.37	1.94	2.95	152	81
9	135	Open	4.86	156	118	0.76	18.28	13.83	43.74	46.59	4.45	2.81	3.51	4.20	170	186
	222	Open	5.50	186	132	0.71	19.10	13.46	40.94	44.82	5.64	3.88	3.30	3.40	170	189
	317	Open	5.68	181	119	0.66	19.52	13.62	39.33	44.06	5.90	4.73	3.07	2.52	168	210
10	258	Open	3.09	120	78	0.65	20.84	15.27	50.89	54.45	5.57	3.56	2.16	2.19	164	183
	310	Open	4.75	110	74	0.67	22.10	18.00	42.51	46.04	4.10	3.53	2.71	2.10	122	180
11	250	Open	10.2	142	119	0.84	18.54	10.52	39.16	43.74	8.02	4.58	1.77	2.60	132	
	314	Open	7.17	133	103	0.78	19.65	11.79	32.64	39.92	7.86	7.24	1.70	1.42	94	
	336	Closed		136			21.50	12.54	36.27	42.98	8.96	6.71	1.52		116	
12	236	Open	4.94	117	65	0.56	22.89	17.93	34.63	38.59	4.96	3.96	2.36	1.64	122	207
	300	Open	4.35	107	94	0.88	22.45	16.60	35.16	40.15	5.55	4.99	1.83	1.88	106	180
	380	Closed		123			24.45	15.46	38.56	45.84	8.99	7.28	1.36		125	198

\* Time in minutes after induction of anesthesia.

† Method of determination of oxygen consumption. "Closed" = spirometer and oxygen. "Open" = collection and analysis of expired air.

‡ Venous blood taken from right auricle in these experiments, from the right ventricle in the remainder.

per 100 cc., blood was taken simultaneously from the right and left pulmonary arteries. The difference in oxygen content was 0.39 cc. per 100 cc.

Table 2 shows the results of serial determinations of cardiac output. In experiments 1 to 3 the exact time at which the first determination followed the



induction of anesthesia is not known, but the interval was less than 180 minutes. It appears that in the barbitalized dog, cardiac output decreases markedly during the first four hours after induction of anesthesia. The expiration of carbon dioxide is extremely variable in such dogs, and figures for cardiac output computed from  $\text{CO}_2$  figures may differ markedly from those computed from  $\text{O}_2$  figures. Respiratory minute volume and the apparent respiratory quotient vary markedly. Whether open or closed respiratory system is used does not appear to alter the observed phenomena. In four of five experiments, arterial  $\text{O}_2$  content varied directly, arterial  $\text{CO}_2$  content inversely with the respiratory minute volume (fig. 1).

DISCUSSION. Several investigators have described the maintenance of discrete streams of blood flow beyond a venous confluence (12) and through the fetal heart (13). It appears that a similar phenomenon occurs in the dog's

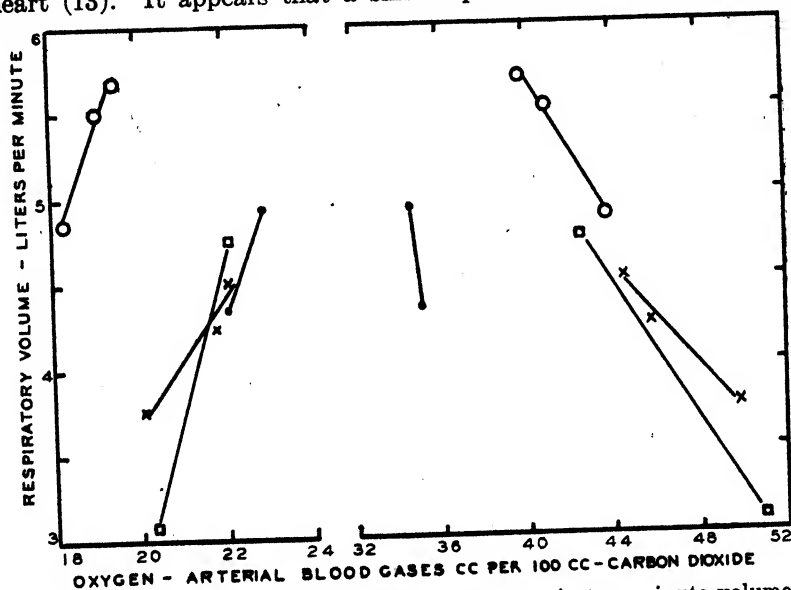


Fig. 1. The relation of arterial blood gas content to respiratory minute volume. Each experiment is identified by a separate symbol.

Right auricle. Blood from the superior vena cava contained less oxygen than blood from the inferior vena cava, and on five of six occasions, blood from the cephalad portion of the right auricle contained less oxygen than blood from the caudad portion. Blood from the right ventricle differed in oxygen content from blood that was taken from the right auricle, containing more as often as less. The difference seen here is greater than that reported for man, when a catheter is introduced into the right ventricle, and subsequently withdrawn into the right auricle (14). However, our observed difference between samples taken from two points in the right auricle is less than this difference reported for man (14).

We do not have data bearing on the question of the degree of mixing of blood in the right ventricle. An undesirable feature of the procedure of ventricular puncture, in our hands, is that some pneumothorax usually results.

The average time that it takes blood to pass from the vena caval-auricular

junction to the pulmonary artery is not known, but is computed as  $2\frac{1}{2}$  seconds, in an anesthetized dog with a heart rate of 160 per minute, a cardiac output of 2400 cc. per minute, and an assumed blood content of right auricle and right ventricle of six times the stroke volume, namely, 90 cc. Since the superior and inferior vena caval blood oxygen content differs considerably, and if there are only  $2\frac{1}{2}$  seconds for blood to become mixed in the right auricle and ventricle, one might question the completeness of mixing of blood in either the right auricle or right ventricle. If the time for blood to pass from the vena caval-auricular junction to the pulmonary artery is prolonged, then one might expect better mixing. This may be the case in the unanesthetized dog in which the normal heart rate is around 70 per minute.

It should be pointed out that with a certain error in the mixed venous blood oxygen content, say 1 cc. per 100 cc., the percentage error in the estimation of the cardiac output is smaller the greater the arteriovenous oxygen difference.

A decrease in cardiac output during the early period of barbitol anesthesia in dogs has previously been reported (15), but the time for approach to a steady state was said to be 90 minutes. Doi (16) saw with the Fick procedure a constant cardiac output in cats under urethane anesthesia, during changes made in the oxygen content of inspired air. Marshall (11, 17) used the Fick procedure in unanesthetized dogs, and stated that serial determinations of cardiac output on the same day showed a variation of 10 per cent or less, but Tappan and Torrey (18) found, with the Fick procedure, decreases of 15 to 35 per cent in successive determinations in morphinized dogs. Green and others (19) have recommended that a control period of three to four or more hours be allowed during anesthesia with barbitol, morphine, etc., on the basis of their observations of progressive changes during that period in hematocrit, oxygen consumption, rectal temperature, and cutaneous blood flow.

Our results show frequent large discrepancies between cardiac outputs as computed from  $O_2$  and  $CO_2$  data. The calculation from  $CO_2$  data may be expected to be more erroneous (20, 21). In the calculation of the cardiac output by the Fick method it is necessary that one measure over a period of several minutes the average rate of  $CO_2$  elimination by the respiratory tract, and the average arteriovenous  $CO_2$  difference over the same period of time. If the respiratory minute volume changes during the above time the alveolar  $CO_2$  concentration, arterial and venous  $CO_2$  content, and the arteriovenous  $CO_2$  difference may change. Thus the arteriovenous difference that one determines at one instant during the above time may not represent the average arteriovenous  $CO_2$  difference and therefore the calculated cardiac output will be in error. Much smaller errors in cardiac output would be expected when oxygen is used in the determination for considerable changes in alveolar oxygen concentration cause little change in the arterial or venous oxygen content, or arteriovenous oxygen difference. The variations seen in the respiratory quotient are also explicable on this basis. Evidence for such changes is given by the values for respiratory minute volume and arterial  $CO_2$  content (see also (5)). Respiratory minute volume and the rate of  $CO_2$  elimination do not seem to show a progressive unidirectional change as is seen in cardiac output.

## SUMMARY

The oxygen content of blood frequently differs when drawn simultaneously from the two venae cavae, from two points in the right auricle, or from the right auricle and right ventricle, in dogs in barbital anesthesia. Estimation of cardiac output in the barbitalized dog by the Fick procedure with blood taken from the right auricle may be in considerable error as a result of obtaining a non-representative sample of mixed venous blood.

There is a progressive decrease in cardiac output during the first four hours of barbital anesthesia in dogs.

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# ABSORPTION, DISTRIBUTION AND EXCRETION OF THIOUREA<sup>1</sup>

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In addition to the recently demonstrated antithyroidal action of thiourea, the use of this substance in measuring renal function (1) and in the estimation of the total body (2-3) water has been considered. In this paper is considered the possible usefulness of the drug in the light of studies dealing with its absorption, distribution and excretion in the body. The investigations on thiourea were performed in conjunction with similar ones dealing with thiouracil (4-6).

**METHODS.** The studies were conducted on rats and man. The rats were of the Wistar strain and were raised in this laboratory.

The methods used for the estimation of thiourea in tissue and body fluids are the ones previously reported for thiouracil (4) and consequently only a few details will be repeated here. The methods are based on Grote's observation (7) that a blue color was formed by substances of a C = S type when treated with a special reagent which is made by the treatment of sodium nitroferricyanide in sodium bicarbonate solution with hydroxylamine hydrochloride followed by bromine. When Grote's reagent is added to thiourea, a blue color is formed and its intensity is estimated in an Evelyn photoelectric colorimeter, using a 620 filter and no. 6 diaphragm. The maximum transmission, as shown by a Hardy recording spectrophotometer, occurs at 600 mμ (fig. 1). When the colorimeter readings of standard solutions, varying in concentration from 0.2 to 10 mgm. per cent, are plotted on semilogarithmic paper, a straight line is obtained. This method has been used in the determination of thiourea in essentially all of the fluids and tissues of the body. When a concentration of thiourea greater than 1 mgm. per cent is present the recovery is almost 100 per cent (table 1). In fact, almost all thiourea can be recovered from the carcasses of rats if the animals are analyzed about one minute after injecting thiourea intravenously (fig. 2).

Many substances, especially compounds with divalent sulfur linked to a single non-metallic element, react with Grote's reagent to give a color. Creatinine in an acid solution or sodium thiocyanate and thiosulphate in neutral or acid solution produce a blue color with the reagent. Therefore, as in the estimation of thiouracil previously reported from this laboratory (4), the pH of the solution to be tested was adjusted to a pH of from 8.5 to 9 before the addition of Grote's reagent. In this pH range no color was developed by potassium sulfide, sodium sulfite, thiocyanate and thiosulphate, uracil, urea, creatinine, cysteine, cystine, glutathione, choline, methionine, thiamine and sulfanilamide when these sub-

<sup>1</sup> This investigation was aided in part by a grant from the Milton Fund of Harvard University.

stances were tested in concentrations equivalent to about 60 mgm. per 100 cc. of thiourea. This concentration was chosen as it is in excess to the quantities of thiourea that we were working with.

In a strongly acid solution, such as a pH of 2, thiourea develops a more intense color than at a pH of 8.5. However, as the pH of Grote's reagent alone is lowered

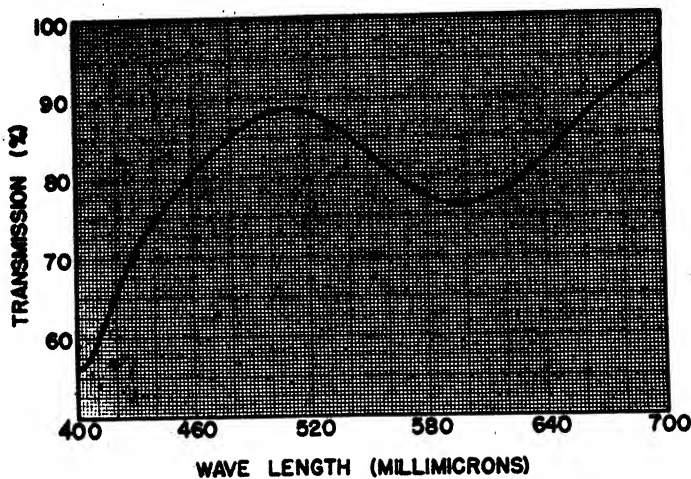


Fig. 1. Absorption curve of the reaction product of Grote's reagent with thiourea as obtained by a Hardy recording spectrophotometer.

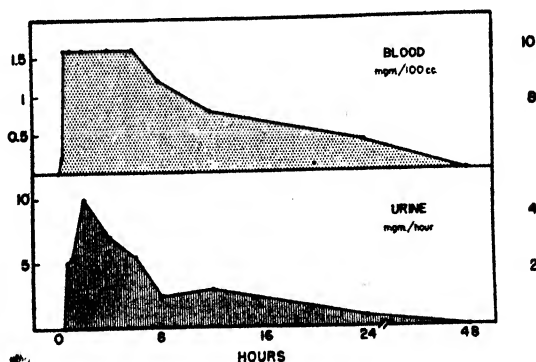


Fig. 2. Note the rapid rate of absorption and excretion of thiourea in a normal individual given 200 mgm. of this substance by mouth.

the more intense is the color. Grote's reagent turns green when made acid. Normal blood and Grote's solution when mixed in equal amounts give a pH of about 8. A maximal color development was found to occur in 15 minutes and it remained stable for at least two and three-quarter hours.

- In the estimation of thiourea in whole blood it is necessary to free it from the red and white cells, otherwise most of the drug remains in the protein precipitate.
- Thiourea is liberated from hemolyzed blood by digestion with trypsin; for the analysis of serum, digestion is unnecessary (see below).

In essentially all of the determinations where protein precipitation was desired, sodium tungstate and copper sulphate were used together, since this method of precipitation was successful in the estimation of thiouracil whereas most of the acid precipitants were unsatisfactory. However, in the determination of thiourea we have used tungstic acid and trichloroacetic acid a few times with satisfactory results.

An aqueous solution of thiourea is almost entirely destroyed within a few minutes by autoclaving or boiling. Therefore the solutions which were injected intravenously in man were made up in physiological saline and sterilized by Berkfeld filtration. Since some of the drug was lost during this procedure it

TABLE 1

*Recovery of thiourea added to blood, urine and tissue suspension*

ADDED	BLOOD		URINE		LIVER	
	Recovered	Per cent recovery	Recovered	Per cent recovery	Recovered	Per cent recovery
<i>mgm./100 cc.</i>	<i>mgm./100 cc.</i>		<i>mgm./100 cc.</i>		<i>mgm./100 cc.</i>	
0.4	0.3	75	0.4	100	0.4	100
0.8	0.7	87	0.8	100	0.8	100
1.6	1.6	100	1.6	100		
2.4	2.2	92	2.4	100		
3.2	3.2	100	3.2	100	3.0	94
4.0	4.0	100	4.0	100	3.8	95
8.0	8.0	100	8.0	100	7.8	97
10.0	10.0	100	10.0	100		
16.0					15.0	94
24.0					23.0	96
32.0					32.0	100
40.0					38.0	95
60.0					60.0	100
100.0					100.0	100

was necessary to estimate the quantity remaining. The solution given intravenously to rats was prepared in this manner immediately before use but was not sterilized.

Estimations of the water content of tissues and fluids were made by subtracting the wet weight from the dry weight of specimens.

**RESULTS.** *Absorption.* That thiourea is very rapidly absorbed from the gastro-intestinal tract has been repeatedly demonstrated in this laboratory by examining the blood and urine at frequent intervals following its ingestion. For example, in a normal fasting individual given a 200 mgm. tablet of thiourea, some drug was found in the blood 15 minutes later (fig. 2) and the maximal concentration was obtained after 30 minutes. Furthermore, an appreciable amount appeared in the urine within 30 minutes.

*Distribution.* The blood cells acquire a greater concentration of thiourea than does the plasma; in these cells the drug becomes bound to protein. The red cells

store a larger total amount than do white cells, but a smaller quantity in each cell. For example, one patient was given 0.4 gram of thiourea three times daily for 2 days and then 1 gram one hour before a blood sample was drawn. The hematocrit showed that 40 per cent of the blood consisted of packed red blood cells, 0.1 per cent white blood cells and 59.9 per cent plasma. The white blood count was 8,700 with a normal differential count, and the red blood count was 4,600,000. The distribution of thiourea, expressed as mgm. per 100 cc. of whole blood, was as follows: plasma, 0.72; blood cells, 1.92; white cells, 0.60; and red cells, 1.20. The same relative distribution was obtained *in vitro* by adding thiourea to normal blood, 5 mgm. per 100 cc., and incubating the mixture at 37°C. for one hour.

Rats given 0.1 per cent of thiourea in the drinking water for several days showed a marked variation in the concentration of this substance in the tissues. Some tissues contained a greater concentration than blood whereas others had less.

*Destruction in the body.* It has been demonstrated (5) that marked destruction of thiouracil takes place in the body, essentially all tissues having the capacity to break down this drug. As shown below, thiourea has a similar fate. Each of 15 rats, fasted for 12 hours and weighing approximately 125 grams, was given 1 cc. of a 500 mgm. per cent solution of thiourea into the femoral vein. Care was taken to ensure that thiourea did not extravasate. Under ether anesthesia the vein was exposed and a 26 gauge needle was used for the injection. After withdrawal of the needle the opening in the vein was cauterized. During the experiment the animals were kept in beakers and any thiourea remaining in the container was washed out and added to the carcass. Five of these animals were killed by a blow on the head one minute after injection, 5 were killed at the end of one hour and five were killed after 3 hours. A fairly uniform suspension of each animal was prepared in the manner previously described (6) and an estimation was made of the total amount of thiourea present in the carcass. The accuracy of this method of analysis is indicated by the results obtained in the 5 animals killed one minute after injection (fig. 3), which indicates that from 88 to 100 per cent, or an average of 96 per cent of the administered drug, was recovered from these animals. After one hour 55 per cent, and after 3 hours 27 per cent, remained in the carcass. Thus the destruction of the drug proceeds rapidly, but not at a uniform rate either in the same or in other rats.

*Distribution of thiourea in relation to body water.* Four male rats were fasted for 12 hours and were given intravenously, in the manner described above, 0.25 cc. of 2 per cent thiourea. Two hours later, under ether anesthesia, about 2 cc. of blood was withdrawn from the aorta and the animal was then killed by a blow on the head. Samples of liver and muscle were taken for analysis. The water and thiourea content of the liver, muscle, blood and carcass were then estimated. The concentration of thiourea in the blood was remarkably constant but was quite variable in the other specimens (table 2). When the amount of thiourea was considered in proportion to the water content of various tissues, the liver was found to contain several times as much thiourea as did muscle and the

concentration in the carcass differed from that in muscle and liver, but was more nearly that of blood. The amount of the drug in the same type of specimen from different animals, e.g., the concentration of thiourea in the liver, also varied a great deal. Presumably, about 40 per cent of the thiourea was destroyed.

In another series of experiments a study was made of the relation of the concentration of thiourea in the plasma and whole blood to the total body water.

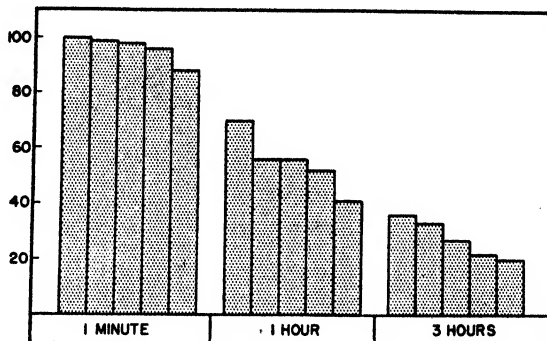


Fig. 3. Recovery of thiourea from whole rats following the injection of 5 mgm. of this chemical intravenously. Each column represents the results of analysis in one rat.

TABLE 2

*The distribution of thiourea in liver, muscle, carcass and blood of rats in relation to water content*

RAT	LIVER				MUSCLE				BLOOD				CARCASS				THIO-UREA TOTAL MGm. TU	EN-TIRE RAT
	Per cent water	Mgm. Tu./100 gm. W.†	Mgm. Tu./100 gm. D.W.‡	Mgm. Tu./100 cc. water	Per cent water	Mgm. Tu./100 gm. W.†	Mgm. Tu./100 gm. D.W.‡	Mgm. Tu./100 cc. water	Per cent water	Mgm. Tu./100 gm. D.W.†	Mgm. Tu./100 cc. water	Mgm. Tu./100 cc. blood	Per cent water	Mgm. Tu./100 gm. W.†	Mgm. Tu./100 gm. D.W.‡	Mgm. Tu./100 cc. water		
A	71	11.5	39.8	16.3	76	4.1	17.4	5.5	78	27	7.0	6	75	4.0	26.6	8.9	2.9	58
B	71	8.7	30.2	12.4	76	2.2	9.4	2.9	83	35	7.2	6	75	4.3	17.2	5.7	3.1	63
C	71	14.8	51.0	20.8	74	1.1	4.3	1.5	79	31	7.6	6	76	5.2	21.6	6.8	3.1	64
D	72	16.4	58.6	14.5	81	0.8	4.0	0.9	83	35	7.2	6	75	3.4	13.6	4.5	2.7	53

\* Tu—Thiourea.

† W.W.—Wet weight.

‡ D.W.—Dry weight.

Ten rats in different states of hydration were used for the investigation. Three rats, classed as "markedly dehydrated" were given no water for 60 hours preceding the test. Four rats, considered as "moderately dehydrated," were given no water for 24 hours before the test. Three rats, designated as "hydrated", were permitted water until the thiourea was injected. None of the animals was given food during the 24 hours before the test. The hydrated animals were given 4 cc. of saline, intravenously, 30 minutes before the thiourea was given. Each of the 10 rats was given 5 mgm. of thiourea, the dehydrated



animals receiving this in 0.5 cc. of saline, while the others received it in 4 cc. of saline. Several of the hydrated animals died of pulmonary edema and some of the others were dyspneic and cyanotic. After 2 hours each of the rats was anesthetized with ether and about 3 cc. of blood was withdrawn from the aorta; the animal was then quickly killed. The gastro-intestinal tract was removed and analyzed separately. A suspension of the carcass was made in the manner previously described and estimations were made of the total water and thiourea content of the carcass. The urine passed during the experiment was collected and analyzed separately. The amount of thiourea in the urine and in the gastro-intestinal tract was relatively insignificant, being less than 5 per cent of the quantity injected.

TABLE 3  
*The distribution of thiourea in relation to the body water*

The distribution of thiourea in relation to the body water.

	PLASMA				WHOLE BLOOD				CARCASS				TOTAL RAT						
	Mgm. Tu per cent plasma	Per cent water	Mgm. Tu 100 gm. dry plasma	Mgm. Tu/100 cc. water	Mgm. Tu per cent whole blood	Per cent water	Mgm. Tu/100 gm. dried blood	Mgm. Tu/100 cc. water	Mgm. Tu re-covered	Per cent water	Mgm. Tu/100 gm. wet wt.	Mgm. Tu/100 gm. dry wt.	Mgm. Tu/100 cc. water	Total mgm. found	Per cent recovery	Wt. gm.	Calculated cc. water	Cc. water actually found	Ratio of calculated* to actual water content
Markedly dehydrated	1.9	89	17.2	2.12	6	69	19	8.6	1.8	70	0.81	2.7	11.5	1.98	40	221	236	154	1.53
	2.0	89	18.1	2.24	6	68	18.7	8.9	3.6	71	1.61	5.54	21.8	3.78	75	218	222	155	1.43
	1.9	88	15.8	2.16	6	68	18	8.4	3.2	70	1.95	6.5	27.6	3.41	68	164	236	114.8	2.05
Moderately dehydrated	2.0	92	25	2.1	6	69	19	8.5	2.94	74	1.61	6.18	21.8	3.12	62	182	238	134.7	1.76
	2.0	92	25	2.1	6	70	19	8.6	3.2	73	1.7	6.34	23.4	3.56	71	187	236	136	1.73
	2.0	91	22.2	2.2	5.8	74	22	7.9	2.7	74	1.49	5.7	20	3.05	61	181	226	134	1.69
	2.0	91	22.2	2.2	6	75	24	8.0	2.45	72	1.35	4.84	18.4	3.57	71	181	226	130	1.74
Hydrated	2.0	92	24	2.2	6	76	25	7.9	2.0	76	0.89	3.7	11.6	2.06	41	225	227	171	1.33
	2.6	92	31	2.8	6	76	25	7.9	2.16	78	1.56	7.1	20	2.22	45	138.5	178	108	1.65
	2.0	95	40	2.1	6	76	25	7.9	2.66	79	2.0	9.52	25.4	2.81	56	132	236	104	2.27

\* Calculated on the basis of the concentration of thiourea in the serum, assuming that thiourea is uniformly distributed throughout the body water.

It may be clearly seen from the data presented in table 3 that the concentration of thiourea in the blood or in the plasma does not indicate the amount of water in the body. Although the water content of the blood was distinctly different in the 3 groups of animals the concentration of thiourea in the plasma was almost identical. The amount of thiourea per 100 cc. of water in the plasma, blood and carcass varied a great deal. The amount of water actually found in the animals was considerably less than the quantity calculated to be present, on the basis of the concentration of thiourea in the plasma, and it was much greater than the amount calculated on the basis of the findings in the blood (table 3).

**Excretion.** Examination of stool specimens saved over a period of 4 days in 2 subjects receiving 0.6 gram of thiourea daily showed no thiourea. The drug is rapidly excreted in the urine, appearing within 30 minutes after ingestion and the maximal rate of excretion occurs within the first one or two hours (fig. 2).

Within 3 days after the cessation of treatment with thiourea this substance has disappeared from the blood and urine of normal individuals. However, with doses of about 0.6 gram daily only about one-third of this amount is excreted in the urine.

When thiourea in doses of about 500 mgm. in saline was injected intravenously and urine specimens were collected frequently it was found in 12 normal individuals that about 3 per cent was excreted in the urine in the first 30 minutes (table 4). Three patients with moderate to severe impairment of renal function excreted an average of only 1 per cent. Moreover, 2 subjects with pronounced Laennec's cirrhosis excreted only 1.1 per cent. The maximal rates of excretion in the normal individuals occurred in the first hour and progressively declined thereafter. Very little was excreted after 24 hours and none after 48 hours. Although the 2 patients with cirrhosis excreted the drug slowly at first, by the end of 24 hours they had an essentially normal excretion; they excreted none

TABLE 4

*Per cent of thiourea excreted in the urine following its injection intravenously in doses of from 400 to 580 mgm.*

SUBJECTS		HOURS						
Type	No.	$\frac{1}{2}$	1	2	4	8	24	48
Normal.....	12	3.5 (2-6)	6.7 (4.7-7.2)	9.2 (7-11)	12.6 (10-15)	22.8 (21-26)	33 (16-42)	35 (31-42)
Nephritic...	3	1.0 (0.9-1.2)		3.1 (2.7-3.5)			6.6 (1 case)	
Cirrhotic....	2	1.1 (1.0-1.2)		6.7 (3-11)			32.5 (30-35)	32.5 (30-35)

after the first 24 hours. The nephritic patients exhibited a marked impairment in the excretion of thiourea.

DISCUSSION. Thiourea has not proved to be very satisfactory in the treatment of thyrotoxicosis, due to the smell, nausea, skin rashes and fever which it not infrequently produces. Although its use as an indicator of the total body water has been considered (2, 3, 8, 9), it is not very satisfactory for this purpose. The diffusion of thiourea throughout the body has been regarded as perhaps similar to that of urea (2), which has been stated to diffuse equally throughout the body. A recent report (11), however, indicates that urea is not distributed uniformly. Moreover, as shown above, there is a marked variation in the proportion of thiourea to the water content in various tissues and body fluids. Furthermore, it is rapidly broken down in the body at an irregular rate. Therefore, the apparent volume of distribution is far in excess of the actual volume of water. Thiourea has been shown to be excreted by the kidneys in a manner similar to the excretion of urea (1, 9). With impaired renal function there is a decreased excretion of thiourea. The specific breakdown products of thiourea have not

been determined. However, following its administration to normal individuals there is an increased excretion of neutral sulfur in the urine (6, 12).

#### SUMMARY

Methods for the estimation of thiourea have been modified for the determination of the concentration of this substance in any of the fluids and tissues of the body.

Thiourea is rapidly absorbed from the gastro-intestinal tract and is rapidly distributed throughout the tissues and fluids of the body; its concentration in the tissues varies widely. The distribution of thiourea correlates poorly with the water content of body fluids and tissues; the apparent volume of distribution far exceeds the actual content of body water. The substance is broken down in the body in a rapid and inconstant manner. No thiourea is excreted in the stools, but it appears in the urine within 30 minutes after ingestion. None of the substance is usually found in the urine 48 hours after cessation of therapy. In patients with nephritis there is a distinct impairment in the excretion of thiourea.

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# THE ELECTROCARDIOGRAPHIC LOCALIZATION OF MYOCARDIAL INFARCTS BY INJURY CURRENTS AND VENTRICULAR EXTRASYSTOLES<sup>1</sup>

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One of the electrocardiographic features of an acute myocardial infarction, the current of injury, may be produced by other types of injury such as burning or by treating the surface of the heart with an isotonic solution of KCl. As far as their influence on the electrocardiogram is concerned, they are indistinguishable, apart from the question of reversibility, creating the appearance of a displacement of the S-T segment which appears to be due to a deviation of the diastolic base-line in the opposite direction (1).

The method of potassium chloride application, which may therefore be considered to have validity regarding the question of localization of injured regions of the heart, does in fact permit geographical localization of the site of injury with some exactness (2, 3). Damage to the right ventricle results in the appearance of a depressed S-T segment which is manifested in lead III if the damage is restricted to the anterior surface, and which develops in lead I if the posterior surface alone is damaged. If the damage extends over both anterior and posterior surfaces of the heart, the electrocardiographic changes are noticeable in both lead I and lead III. Left ventricular damage, on the other hand, results in elevation of the S-T segment. Anterior left ventricular damage influences lead I, and posterior left ventricular damage becomes apparent in lead III.

Application of KCl to the anterior surface of the heart, overlapping the septum and involving both right and left ventricles, will give results predictable from the simpler situation above; anterior right ventricular damage will be manifested in lead III as depression of the S-T segment, while the damage to the anterior left ventricle will produce an elevation of the S-T segment in lead I. By similar reasoning it can be calculated that posterior damage, affecting both ventricles, will produce a depression of the S-T segment in lead I and an elevation of the segment in lead III, and the prediction is borne out experimentally.

The extension of these facts to the interpretation of the damage produced by occlusion of coronary arteries meets with certain theoretical objections. One of these concerns the extent of an infarct as compared with the injury following the application of KCl. If the endocardium were also involved, according to one theoretical treatment of the influence of an injury current, quite different results might be obtained than if the outer surface alone were involved. Another account has it that the electrocardiographic signs of an infarct are related to

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the specific muscle-bundle deprived of blood, and bear no simple geographical relation to the surface of the heart supplied by the artery occluded (4). Results of experimental ligation of selected coronary arteries are not completely in agreement (5, 6), for a number of reasons related to the selection of arteries to be ligated and the variability in the regions supplied by each artery, the leads used in recording, and the condition of the lungs and the chest opening during recording (7, 8). In any event, there is no clearcut recognition of the four fundamental localizing patterns that can be made out with the potassium method.

A further aid in localization is afforded by study of the ventricular extrasystoles that arise when an area of the heart is damaged by coronary ligation. These extrasystoles, which are almost invariably present in the dog, undoubtedly arise from within or around the damaged area, as has been most clearly demonstrated by Harris and his co-workers (9). Since it is possible to make an accurate localization of the site of origin of a ventricular extrasystole in the dog, an additional electrocardiographic localizing sign becomes available (10). The present report concerns the study of electrocardiographic localization by both methods simultaneously, and it will be seen that the two methods are in agreement. It might be pointed out explicitly that the experiments reported here deal only with the deviations of the S-T segment associated with the persistence of an injury current, and do not concern, although they are not incompatible with, the localization of healing infarcts by QRST changes.

**METHOD.** Thirteen dogs were employed. They were deeply anesthetized with Dial, and the heart exposed as previously described. Ischemia was produced in four regions of the heart, *a*, right lateral; *b*, left lateral; *c*, anterior; *d*, posterior; by occlusion of the appropriate descending coronary arteries supplying those regions. In the right and left lateral positions *a* and *b* the regions of ischemia were restricted to a single ventricle and could be recognized readily as sharply-circumscribed blue areas. In *c* and *d*, on the other hand, occlusion of vessels descending at the septum, both anterior and posterior, produced well-marked ischemic regions in both the right and the left ventricle on each side of the septum. The lungs were expanded, the chest wall closed with clips, and the artificial respiration suspended, so that the animal breathed spontaneously. Ventricular extrasystoles appeared after a variable interval, were first observed in the cardioscope and then were recorded in leads I and III simultaneously by means of a Sanborn Tribeam. The same record showed the pattern of S-T segment deviation in the supraventricular complexes together with the configuration of the ventricular extrasystoles.

**RESULTS.** *A. Ischemia of lateral region of the right ventricle.* In five experiments ischemia was produced in and around the center of the right ventricle (fig. 1 A, B, C). The characteristic electrocardiographic pattern of exclusively right ventricular damage appeared, namely, depression of the S-T segment in both lead I and lead III. The configuration of the associated ventricular extrasystoles which appeared spontaneously indicates that their site of origin was in the lateral region of the right ventricle because the initial deflection is exclusively upright in both leads I and III.

*B. Left lateral ischemia.* Records of left lateral ischemia from five experiments are also shown (fig. 2 A, B, C and fig. 3 A, B, C). In each instance the supraventricular complexes showed the S-T segment elevation characteristic of damage to the left ventricle. These were present in both leads I and III, indicating that both anterior and posterior parts of the ventricle were involved. The accompanying ventricular extrasystoles show a variety of origins, all of them, however, from within the left ventricle. Thus in figure 2 A the presence of a  $QS_1$  and an  $RS_3$  indicates that the extrasystole originated in the anterior portion of the left ventricle. Figure 2 B shows an extrasystole with a  $QS_1$  and a small w-shaped  $QRS_3$ , from which it can be inferred that the extrasystole arose near the apex in the anterior portion of the left ventricle. The  $QS_1$  and  $RS_3$



Fig. 1

Fig. 2

Fig. 1 A. April 28, 1943. Eight minutes after occlusion of the right coronary artery near the right lateral margin of the heart. This produced a large area of ischemia extending from base to apex at the lateral margin of the right ventricle which did not involve the left ventricle of the apex.

B. April 29, 1943. Forty minutes after occlusion of the right coronary artery similar to A.

C. August 19, 1943. Four hours after an occlusion of the right coronary artery similar to A and B.

Fig. 2. April 27, 1943. Occlusion of a branch of the left circumflex artery descending to the apex along the lateral margin of the left ventricle. A, B and C: taken 6, 7 and 8 minutes after occlusion.

of the extrasystole in figure 2 C localize its site of origin in the anterior portion of the left ventricle, while the large amplitude of both leads I and III suggests that this extrasystole arose nearer the base than did those in 2 A or B. In figure 3 B and C are two short runs of ventricular tachycardia, from foci changing from anterior left to left lateral, as evidenced by the persistent  $QS_1$  and a shift from  $RS_3$  to  $QS_3$ .

*C. Anterior septal ischemia.* Ligation of the anterior descending artery produced an ischemic area overlying the anterior portions of both right and left ventricles. The anterior left ventricular damage is recorded in lead I as an elevation of the S-T segment, while the damage to the anterior right ventricle is recorded in lead III as a depression of the S-T segment (fig. 4 A, B, C). A typical anterior septal pattern is seen in the extrasystole of figure 4 A, which shows a characteristic  $QS_1$  and  $R_3$ . The extrasystole of figure 4 B arises somewhat to the left of the septum since it shows a  $QS_1$  and an  $RS_3$ . On the other

hand, the extrasystole of figure 4 C arises to the right of the anterior septum, since it exhibits a  $QR_1$  and  $R_3$ .

*D. Posterior septal ischemia.* Ligature of the arteries descending along the posterior septum produced a zone of ischemia covering both right and left ventricles in and around the region of the posterior septum. The right posterior damage is recorded in lead I in the characteristic depression of the S-T segment while the damage to the posterior left ventricle leads to the development in lead III of an elevation of the S-T segment (fig. 5 A, B). The accompanying extrasystoles in both A and B show an  $RS_1$  and a  $QS_3$  which locate their origin to the left of the posterior septum.

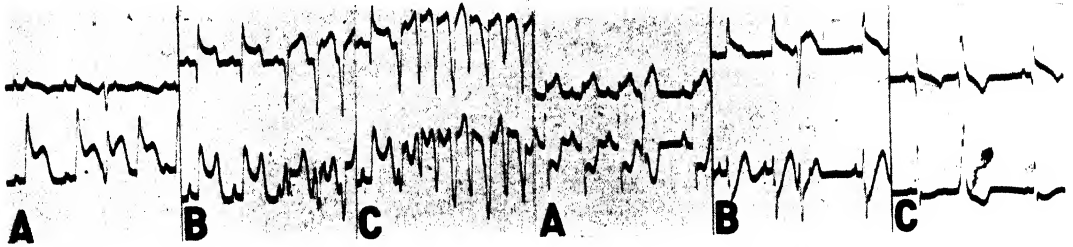


Fig. 3

Fig. 4

Fig. 3 A. April 28, 1943. Occlusion of the left lateral descending branch of the left circumflex for 25 minutes.

B and C. April 23, 1943. Continuous record taken 5 minutes after ligation of the left lateral descending branch of the left circumflex. In all experiments there was a very clearly defined area of ischemia which involved the lateral portion of the left ventricle as well as the apex, but did not encroach upon the right ventricle.

Fig. 4 A. August 26, 1943. Occlusion of the anterior descending artery near the base for 11 minutes.

B. April 23, 1943. Four minutes after ligation of the anterior descending artery near the base.

C. April 25, 1943. Twenty-two minutes after occlusion of the anterior descending artery near the base. In all of these experiments the zone of ischemia involved the anterior surface of the heart, and included portions of both right and left ventricles.

In many records the displacement of the S-T segment seen in the supraventricular complex is also exhibited by the extrasystole.

In a number of experiments the occluded artery was released before permanent damage was produced, and the electrocardiogram subsequently returned to its configuration in the control. A second area was then rendered ischemic, and when extrasystoles appeared it was found that they arose in the new area of ischemia.

**DISCUSSION.** The results of the experiments reported here indicate that damage to the heart produced by occlusion of coronary arteries may be localized by means of the displacement of the S-T segment. The patterns so produced follow the simple geographical distribution of the occluded vessels, as indicated by the general course and direction of the artery, and by the region which becomes cyanotic when the artery is occluded. As far as the S-T segment changes are

concerned, they follow the very same pattern produced by surface applications of potassium chloride.

The extrasystoles which arise in the zone of damage indicate their origin by their configuration in leads I and III, and in every instance there was complete agreement between all four localizing factors: 1, the position and course of the artery occluded; 2, the region of the heart which became cyanotic when the artery was ligated; 3, the pattern of S-T deviation, and 4, the configuration of the ventricular extrasystoles.

Insofar as the dog is concerned, therefore, and it is the animal employed in the majority of previous experimental studies on this topic, four localizing patterns may be made out, being combinations of two basic patterns. Injury to the right ventricle produces an apparent depression of the S-T segment, which is registered in lead I if the damage is to the posterior part of that ventricle.

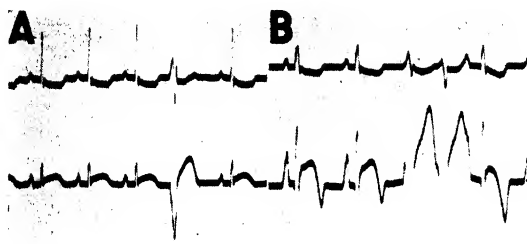


Fig. 5 A. April 29, 1943. Terminal branches of both right and left coronary arteries descended from the base toward the apex near the posterior septum. They were both ligated, giving a single area of ischemia covering the septum and portions of both right and left ventricles. The record was taken 22 minutes after occlusion.

B. August 20, 1943. Posterior descending terminations of right and left coronary arteries tied as in A, and the record B taken 4 hours later.

If the damage is anterior, lead III is involved. Left ventricular damage produces an elevation of the S-T segment noticeable in lead I if the anterior part is damaged and in lead III when the posterior portion is the site of damage. When the S-T segment is deviated in opposite directions in leads I and III, both right and left ventricles have been damaged by an injury involving the septum and adjacent portions of the two ventricles. Elevation in lead I and depression in lead III indicate anterior damage, while depression in lead I and elevation in lead III denote posterior damage.

These findings are in accord with the usual practice in interpreting the electrocardiographic signs of infarction in man, in so far as a distinction between anterior and posterior damage is made. They are equally in accord with earlier observations on experimental animals in which the same distinction could be made (5, 6). In clinical practice as well as in animal experiments the contribution of right ventricular damage to the patterns described has, however, not received adequate recognition, apart from the work of Barnes and Mann (6), who concluded that "infarction in one ventricle produces electrocardiographic effects which are characteristic and opposed to effects produced by the other ventricle."



This has been due in great part to the fact that pathological changes in the myocardium of the right ventricle are not often found at post-mortem examination. Inasmuch as the injury current resulting from ischemia may be followed by complete recovery, as some of these experiments show, it is more than likely that the zone of ischemia which gives rise to S-T segment displacement after coronary occlusion is much more extensive than the region of irreversible damage. This must be particularly true of the right ventricle, where the lesser work load might permit greater opportunity for recovery through collateral circulation. This may be the reason why patterns, which from these experiments would be interpreted as indicating both right and left ventricular injury, have been reported in cases which on autopsy showed no substantial right ventricular damage.

Barnes has observed that, when the injury was carefully limited to the left ventricle, only elevation of the S-T segment resulted. These experiments, which confirm his observations on exclusively left ventricular localization, add the concept of the contribution to the electrocardiogram of right ventricular damage. When such damage is restricted to the right ventricle, only depression of the S-T segment is seen in any of the standard limb leads. When it is combined with damage to adjacent regions of the left ventricle, leads I and III show S-T segment changes in opposite directions. Whether in combination with S-T elevation or alone, S-T depression following ligation of coronary arteries indicates damage to the right ventricle.

Study of the configuration of ventricular extrasystoles that arise spontaneously after occlusion of coronary arteries offers an additional means of localizing the site of injury. The configurations in leads I and III of the QRS portions of the complexes, as well as the pattern of displacement of the S-T segment may be utilized. The former localizes the origin of the extrasystoles, which in confirmation of Harris and co-workers (9) is demonstrated to lie within the damaged area, while the latter localizes the injury current, as does the same change in the supraventricular beat.

#### SUMMARY AND CONCLUSION

1. Myocardial damage was produced in four regions of the dog heart by means of appropriate ligation of coronary arteries. These were *a*, right and left ventricles exclusively, and *b*, anterior and posterior regions involving adjacent portions of the right and left ventricles.

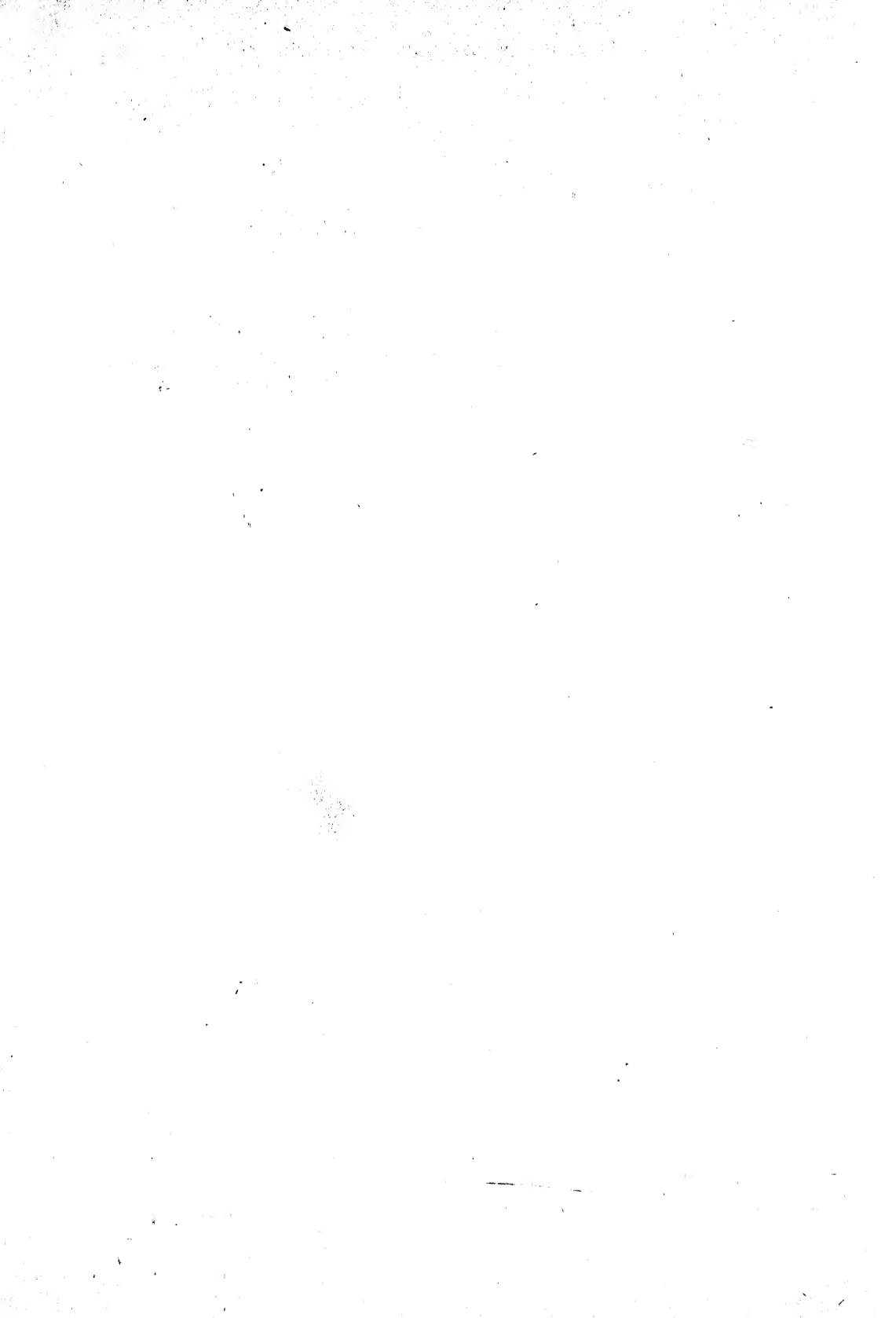
2. The changes in the S-T segment of the electrocardiogram in leads I and III were the same as produced by KCl injury in the same regions, namely, *a*, depressed S-T segment with right ventricular damage; *b*, elevated S-T segment with left ventricular damage; *c*, elevation of S-T<sub>1</sub> and depression of S-T<sub>3</sub> with anterior damage, and *d*, depression of S-T<sub>1</sub> and elevation of S-T<sub>3</sub> with posterior damage.

3. The ventricular extrasystoles which appeared spontaneously after coronary occlusion showed configurations which indicated that they originated within the damaged area. They showed S-T segment changes which were the same

as in the supraventricular complexes. Their configuration in the electrocardiogram may therefore serve as a localizing sign in myocardial damage.

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